JOURNAL OF DAIRY SCIENCE

MARCH, 1956

Features in this issue:

Important happenings in the industry

Recommended policies for a sound dairy program

The first of a series of articles on cheese manufacture

Fourteen recent studies of industry problems

ANNUAL MEETING
June 19-21, '56
STORRS, CONN.

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Manuscripts for publication in the Journal of Dairy Science should be submitted to the editor, P. H. Tracy, at the address above. See January, 1954, issue, page 103, for information concerning the preparation of manuscripts, or send for reprint, "Information for Authors."

Advertising

Advertising copy, insertion orders, contracts, and requests for information relating to advertising in the Journal should be addressed to the editor, P. H. Tracy, at the above address. Advertising plates should be sent directly to The Garrard Press, 119-123 West Park Ave., Champaign, Ill.

Changes of Address

Changes of mailing addresses, post office notices of undeliverable copies, inquiries about copies lost in the mail, requests for back copies, and all other matters pertaining to the mailing list should be sent to the secretary-treasurer, H. F. Judkins, American Dairy Science Association, 32 Ridgeway Circle, White Plains, N. Y.

Subscriptions and Dues

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Subscriptions for the British Isles and the British Empire should be ordered through our agents: Messrs. Bailliere, Tindall and Cox, 7 and 8 Henrietta Street, Covent Garden, London, W. C. 2, England. Subscriptions for Australia should be sent to our agent: John H. Bryant, Herbert Street, St. Leonards, N.S.W., Australia.

Membership dues in the Association are $10.00 a year and should be sent to the secretary-treasurer at the address above. Renewals should be made not later than December 1 each year.

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PEOPLE and EVENTS
in the Dairy Science World

The Dairy Products Improvement Institute Holds Annual Meeting

The ninth annual meeting of the Dairy Products Improvement Institute was held Feb. 16, 1956, in the Hotel Commodore, New York City. The Institute's newly elected president, A. C. FISHER, of the General Ice Cream Corporation, Schenectady, presided during the main session in the afternoon, which was attended by approximately 175 persons from industry, regulatory and educational fields, and the press. After the luncheon the Institute's retiring president, W. A. WENTWORTH, The Borden Company, New York City, presented a noteworthy address on "Cooperation in Product Quality."

The keynote of the session was the panel discussion of the Institute's quality control publication, "Requirements for the Sanitary Production of Sweet Cream and Milk for Manufacturing," which was introduced at this meeting. E. J. ROBERTS of the Breyer Ice Cream Co., Philadelphia, discussed the principles and policies underlying these requirements, and A. C. FAY of H. P. Hood & Sons, Boston, the newly elected vice-president of the Institute, reviewed the procedures and standards presented in the publication. The final speaker for the afternoon was J. A. STALBIRD, chief of the Milk and Restaurant Sanitation Division, New York State Department of Health, Albany, who gave an analytical review of the publication.

R. C. HIBBEN, Intern. Assoc. of Ice Cream Manufacturers, was reelected treasurer and A. C. DAHLBERG, Cornell University, secretary and advisor to the board. D. H. RACE will continue to serve as field director.

Complimentary copies of the "Requirements for the Sanitary Production of Sweet Cream and Milk for Manufacturing," which is published in two parts, the General Statement, and the Operating Manual, were distributed to those attending the meeting. President Fisher announced that the General Statement is available without charge to all who may desire copies, and the Operating Manual will also be distributed free of charge to regulatory officials, educators, libraries, milk market administrators, Institute members, and the press. Others may obtain the Operating Manual at a cost of $2.00 per copy.

Copies of the papers presented and the General Statement and Operating Manual are available at the Institute office, 302 East State Street, Ithaca, N. Y.

Recent Contributions Made to Dairy Fund

The Dairy Remembrance Fund has received 21 new memorial contributions and six others honoring living persons for their contributions to the dairy industry. The honorary contributions were made for the following: C. D. DAHLE of Penn State Univ.; E. S. GUTHRIE of Cornell Univ.; D. J. HANKINSON of the Univ. of Massachusetts; O. E. REED of the U. S. Dairy Assoc., Washington; CHARLES FLOUNDERS of Miller Flounders Co., Chester, Pa.; and A. M. LEMESSURIER, former president, secretary, and treasurer of the Assoc. of Ice Cream Manufacturers of New York state.

The contributions will be allotted to activities that the Dairy Remembrance Fund board of directors feel is constructive to the further development of the dairy industry.

Kommerstad Named Diversey Salesman of the Year

W. E. NOYES, vice-president in charge of sales, has announced the selection of R. J. KOMMERSTAD as Salesman of the Year for the Diversey Corporation. According to Mr. Noyes, "Mr. Kommerstad's selection is a tribute to his conscientious fulfillment of duties to both community and company." The award carries with it an all-expense paid tour of the Caribbean for Mr. and Mrs. Kommerstad. Kommerstad joined Diversey in January, 1946.

Southern Division of A.D.S.A. Meets in Atlanta

The 29th annual meeting of the southern division of the A.D.S.A. was held at Atlanta, Feb. 6-8. President King of Clemson, S. C. presided. Forty papers were presented. At the banquet C. E. WYLIE of the Univ. of Tennessee was presented the honors award for 1955-56. The banquet speaker was HERMAN TALMADGE, former governor of Georgia.

The new officers are H. C. OLSON, Oklahoma, president, K. M. AUTREY, Alabama, vice-president, and J. W. POU, North Carolina, secretary-treasurer. The 1957 meeting will be held in Birmingham.

Winter is a good time to inspect the electrical wiring for signs of deterioration. Replace rotten romex in dairy barns with a moisture resistant type. The words, "In God we trust," were engraved on pennies for the benefit of those who use them as fuses. An ample supply of fuses of the right size should be on hand at all times.

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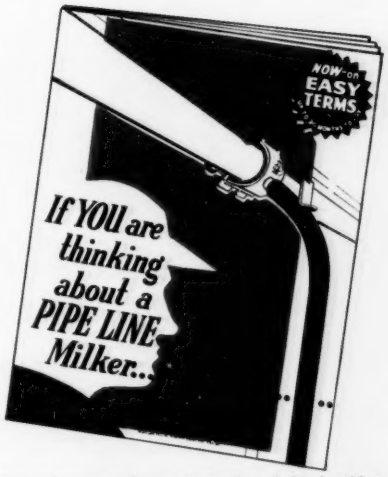

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Texas Staff Appointments

The following new additions to the staff at Texas A. & M. College have been announced by I. W. RUPEL, head of the Dairy Husbandry Dept.: M. A. BROWN, instructor; JOHN KAPSALIS, graduate teaching assistant; CHARUDATTA MULAY, research assistant; R. R. HARRIS, research assistant; and T. J. OUSLEY, creamery superintendent.

Dairyman to Try for U. S. Senate

ELMER DEETZ, the Oregon dairyman who was given credit for the removal of state milk controls, has filed for Republican nomination for the U. S. Senate. He will be pitted against Senator Morse. Deetz was involved in a long series of legal battles over his attempts to sell jug milk at prices lower than those fixed by state regulation. He carried his battle to the people and won not only this issue but a seat in the state senate.

Poulton Joins Maine Staff

BRUCE R. POULTON has accepted an appointment as associate professor in the Dept. of Animal Industry, effective July 1. Mr. Poulton did his undergraduate work at Princeton and Rutgers and will complete his doctorate in animal nutrition and endocrinology at Rutgers, where he is now assistant professor of dairy husbandry.

Manitoba Dairy Group Meets

The 71st annual convention of the Manitoba Dairy Assoc. and Dairy Manufacturers' Assoc. of Manitoba was held at Winnipeg Feb. 21-23. A total of 660 persons (200 manufacturing, 460 production) attended the meeting. P. B. DE LA GRODAY, manager of Palm Dairies, Winnipeg, was elected president for the coming year. C. H. P. KILLICK has served as secretary-treasurer of the group for a number of years.

Guest speakers from the states included GAIL SMITH, J. B. Ford Co., Wyandotte, Mich., E. E. HEIZER, Univ. of Wisconsin, and P. H. TRACY, Univ. of Illinois.

Dairy Statistics from Denmark and Sweden

G. H. WILSTER of Oregon State College, who is on a lecture tour in Denmark, Germany, and Austria, recently sent some interesting information on milk prices as he found them in Copenhagen and Stockholm, and some Danish dairy statistics.

Copenhagen

1 liter milk in store 69 øre
(1 liter = 1.057 qt.; 7 øre = 1 cent)

1 later milk delivered 73 øre

Fat in milk 3.5%

Price to producers 54.18 øre per kilogram 4.2% milk, delivered at Copenhagen, transportation at 2 øre per kilogram included. (1 kg. = 2.2 lb.)

Differential 0.43 øre per kilogram for each 0.05% fat.

Retail store profit 13.3% of sale price.

Stockholm, The Milk Central

1 liter milk, excl. bottle 74.6 Danish øre, in store

1 liter milk not in bottle 68 Danish øre, in store

No milk delivered by organization, except in outlying areas where additional charge is 5.33 øre

Fat in milk 3%

Price to producers 48 Danish øre per kg. 3.5% milk plus 3 øre per kg. fall increase. Differential 0.93 per 0.1% fat.

85% of milk in glass bottles or in Tetra Pak paper.

Retail store profit 12.7% of sale price.

These data should be carefully considered. A person should consider income, taxes, etc. in making an appraisal.

(Continued on page 8)

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is part of an extensive campaign in farm and dairy magazines telling dairymen the many advantages of clean, sanitized equipment, thus giving a valuable "assist" to your own activities.

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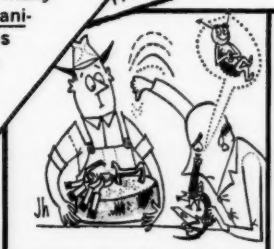

Milkstone deposits in utensils and equipment provide a breeding place for bacteria . . . prevent complete dairy sanitation.

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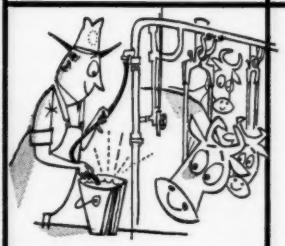

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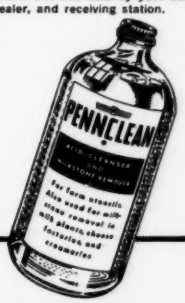

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(Continued from page 6)

The 1954 Dairy Statistics for Denmark are:

	Kg.
Total milk production	5,394,000,000
Milk production per cow	3,558
Milk received at dairy plants and milk retailed by producers	4,994,000,000

Uses:	*Kg.*
For sale as cream	208,000,000
As Market milk, cities	372,000,000
As Market milk, used by producers	155,000,000
For Butter manufactured	3,710,000,000
For Cheese manufactured	551,000,000
For condensed and dry milk	134,000,000
For export of whole milk	19,000,000

Total number of dairy plants	1,484
Total number of cooperative plants	1,258
Total number members of cooperative plants	172,700
Annual turnover per cooperative plant	1,584,000 kroner

7 kroner = 1 dollar

Operating cost per 1,000 kg. milk received	50 kroner
Estimated population Jan. 1, 1955	4,424,000
Of this 2,249,300 in rural districts	

Production of Animal Products in Denmark, 1954

	Kg.
Milk	5,394,000,000
Butter	181,000,000
Cheese	81,000,000
Casein	2,500,000
Condensed milk	27,135,000
Dry milk	11,539,000
Ice cream	14,198,000 liters
Beef and veal	203,200,000
Pork and bacon	524,400,000
Lard	23,200,000
Horse flesh	17,500,000
Mutton and lamb	1,100,000
Poultry	25,000,000
Eggs	142,400,000

In July 1954 there were 1,505,333 dairy cows and 1,579,237 heifers and calves.

Of the cows
- 1,045,751 were Red Danish
- 238,916 were Black & White Danish
- 117,250 were Jersey
- 54,503 were Shorthorn
- 8,101 were of other breeds
- 26,706 were of crosses
- 14,106 were of unknown breeds

New Representatives for Pennsalt

W. J. SMITH will represent the Pennsylvania Salt Mfg. Company's B-K Dept. in Louisiana and southern Mississippi. He received his technical training at Mississippi State Univ. and was formerly associated with the Meyer Blanke Co. and Brooke Supply Co.

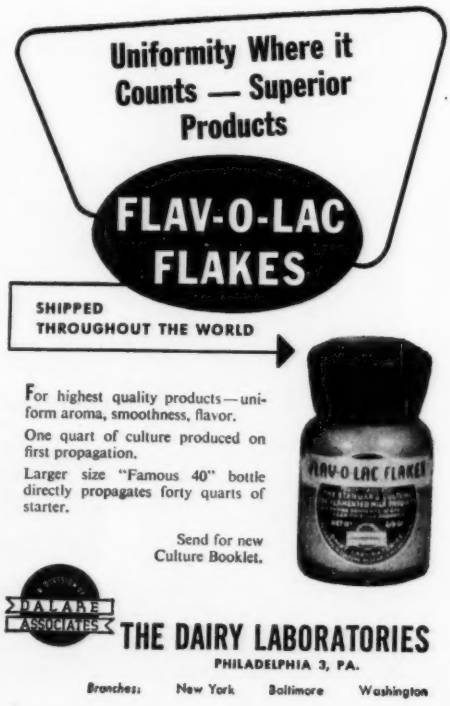
K. D. BUCHEIT, a graduate of the Univ. of Texas, has been appointed sales-service representative in North Texas.

Fairmont Expands Further

The Fairmont Foods Co. of Omaha, Neb., has acquired the Lily Brook Farms, Inc. and the Bowmansville Creamery, both of Bowmansville, N. Y.

R. P. JOSLIN, formerly senior research chemist with the Monsanto Chem. Co. of Dayton, Ohio, has been made production research manager of the Fairmont Foods Co. Joslin received a B.S. degree in chemical engineering at Northeastern Univ. in Boston and his Ph.D. degree in food technology at M.I.T.

JEAN FINEGAN, for a number of years an account executive with Benton and Bowles, a New York advertising agency, has been appointed advertising and publicity manager for Fairmont.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4 An economical means for building up higher total solids content, FRODEX offers other advantages as well: FRODEX will not impair flavor with excessive sweetness; inhibits formation of large ice crystals; contributes to ease of obtaining over-run; prevents crusting and bleeding; will not depress freezing point, and improves melt-down.

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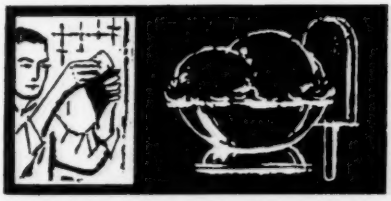

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Supreme Court Refuses Wisconsin Plea

The U. S. Supreme Court has refused the petition of the State of Wisconsin to file a suit challenging the validity of the Tennessee milk price law, which allows producers in that state to sell below a prevailing price and subjects shippers from other states to prosecution if they sell below the prevailing figure. The state of Tennessee opposed the petition on the basis that they had done nothing harmful to the dairy industry of Wisconsin.

Neathery Takes Georgia Post

M. W. NEATHERY, formerly on the staff of the Univ. of Tennessee, has taken a position with the Georgia Mountain Branch Experiment Station at Blairsville.

Martin Lawritson Dies Suddenly

M. N. LAWRITSON, 61, a member of the Univ. of Nebraska agricultural extension staff and a noted dairy cattle judge, died Feb. 5 after a short illness. He received his B.S. degree from the Univ. of Nebraska in 1919. From 1920 to 1924 he was with the dairy extension staff of the University. From 1924 to 1934 he was with the Nebraska Dairy Development Society and for a short time was with the Co-op Milk Assoc. of Lincoln.

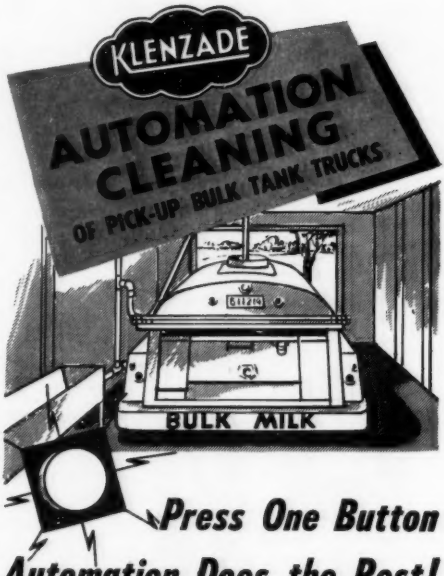
In 1934, Mr. Lawritson rejoined the extension staff. He was recognized nationally as a dairy cattle judge and last fall judged a show in Cuba. He was a pioneer in working with the various purebred dairy cattle associations in Nebraska and aided with the development of 4-H dairy clubs.

He was a member of First Plymouth Church, Alpha Zeta, Epsilon Sigma Phi, Dairy Shrine Club, a charter member of the Nebraska chapter of Alpha Gamma Rho, and a Mason and was recently made an honorary member of the Omaha Chamber of Commerce for his work with 4-H dairy clubs. Surviving are his widow and two children.

Resolution Passed by Purebred Dairy Cattle Assoc.

WHEREAS, The American Dairy Science Association is continually devoting its full efforts to improving the dairy industry, therefore be it

Resolved, That the Purebred Dairy Cattle Association at its meeting today, January 20, 1956, recognize and express its sincere appreciation for the excellent cooperation of this organization in working with the purebred dairy cattle recording societies and the breeders of purebred cattle.



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Chemical Congress to Be Held in Germany in 1958

The ACHEMA 1958, 12th Chemical Apparatus and Equipment Congress and Exhibition, will be held in Frankfurt am Main during the period of May 31 to June 8, 1958. The meeting will be held on the Exhibition Grounds of the City of Frankfurt where all ACHEMA Congresses have been held since 1937. The steadily increasing importance of these exhibitions is very closely linked with the constant increase in the importance of chemical engineering. The European Convention for Chemical Engineering will be held in conjunction with the ACHEMA. The European Federation for Chemical Engineering intends to hold its Second European Chemical Engineering Congress in connection with the European Convention. This latter Congress will, in all probability, be held in Brussels.

News Items from Virginia

G. C. GRAF, head of V.P.I.'s Dept. of Dairy Science, was a principal speaker on the programs of the conventions of the Virginia Dairy Products Assoc. and the Virginia State Dairymen's Assoc., held in Roanoke in January. His topics were "The Department of Dairy Science and You" and "The Current Dairy Situation and Outlook for Virginia." Dr. Graf was reelected director of the Dairymen's Association.

The Dept. of Dairy Science is converting to all bulk handling of raw milk in its barns and dairy plant. Bulk milk tanks have been installed in the three milking barns and a modified tanker will be used to truck the milk from the barns to the V.P.I. dairy processing plant.

N. R. THOMPSON, associate professor of dairy science, recently completed the requirements for his Ph.D. degree at Michigan State Univ. The title of his thesis is "Estimates of Genetic Progress in the Development of the American Red Danish Cattle." Dr. Thompson conducts research on dairy cattle breeding at V.P.I.

V.P.I. received its ninth consecutive Progressive Breeders' Award on the performance of its Holstein herd. The herd averaged 12,626 lb. of milk and 467 lb. of fat for 56 cows. The classification average was 82.0. One of its bulls, Vineland Peanut Ormsby Governor, was also certified as a Gold Medal Proved Sire.

Nord to Administer Shreveport Market

JOHN NORD, for several years connected with the federal milk market order of Chicago, has been placed in charge of the newly created federal order of Shreveport and Central Mississippi. Headquarters will be at Jackson, Miss.

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* In view of numerous requests, a reprint has been issued of the review article in Vol. 23, No. 3 (1955) by D. H. L. Rollinson, "Hereditary factors affecting reproductive efficiency in cattle," priced 2s, 6d. ($0.35).

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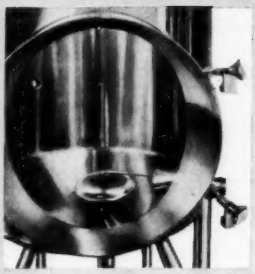

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STUDENT CHAPTER NEWS

A section devoted to the activities of dairy students
E. L. THOMAS, *Editor*

Babcock Dairy Science Club Activities

The Babcock Dairy Science Club of the Univ. of Wisconsin elected officers for 1956 at its December meeting, with 22 members present. CHARLES BRICK, a former student and now production manager of Borden's Madison plant, spoke on the topic "What is expected of us when we get on a job?"

At its January meeting, plans were made for the coming semester's activities. These include organizing the Dairy Products Judging Team and selling refreshments at the Dairy Manufacturers Conference, which will be held on the campus March 13-14.

Minnesota Honors Teams

Members of the Minnesota Dairy Science Club served hot lunches to 400 guests attending Farm and Home Week activities on the St. Paul campus, Jan. 11-12. This is an annual activity of the club and one of its major sources of income.

The annual winter judging contests, sponsored by student organizations of the College of Agriculture, were held during the first week in February. The Dairy Products and Dairy Cattle Judging Contests were conducted by members of the 1955 judging teams. Contest winners were presented awards at a student-faculty awards assembly on Feb. 14. An additional feature of the assembly was the presentation of "M" pins to members of 1955 judging teams and recognition of team members of 10, 20, and 30 years ago.

Rhode Island Students Honored

EARL S. FEINMAN of Portsmouth, R. I., and LEO W. MINISCE of Narragansett, were granted Charles H. Hood Dairy Foundation Awards of $250 each for diligent effort and outstanding scholastic achievement while majoring in animal and dairy husbandry at the Univ. of Rhode Island.

VERNON W. TUXBURY of Adamsville, R. I., was granted the Virginia Dare Award, given annually to a dairy technology senior for excellence in scholastic achievement and its practical application in flavor discrimination and ice cream manufacture.

Rutgers Dairy Science Club News

The Dairy Club recently served luncheons to a Dairy Manufacturers' Conference and to a Dairy Fieldman's Conference, which were sponsored by the Dairy Department. A net profit of $143.00 was realized.

The club members are currently working with the preparation of a yearbook. It is hoped that the copy will be ready for the printers by March. This is a new venture for the club.

Recent speakers at the club meetings have been Dr. Anderson of the Rutgers Farm Crops Department, and also a representative of a large commercial chemical and pharmaceutical company. The former told of his experiences in establishing a corn breeding program in Indonesia, and the latter spoke of the role of drugs in the breeding and raising of livestock. Both talks were illustrated with slides.

Report on ACS Student Affiliate Activities

An interesting and informative discussion of the student affiliate program of the American Chemical Society has been presented by B. R. STANERSON in the Jan. 9, 1956, issue of *Chemical and Engineering News*, pages 148-150. The author emphasizes the importance of chapter activities in preparing the student for his future professional career. Some of the unique and most successful programs organized by affiliate chapters are described.

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JAMES P. GRACY—Enzymatic hydrolysis of lactose for ice cream mix. Univ. of Tennessee, Knoxville.

MILTON W. NEATHERY—The determination of body water in cattle by the use of antipyrine. Univ. of Tennessee, Knoxville.

FRANK E. PINKERTON—The influence of ionizable and nonionizable compounds in milk upon its freezing point. Texas A. & M. College, College Station.

BEN T. THROOP—The identity and probable significance of some streptococci producing atypical Hotis reactions. Univ. of Tennessee, Knoxville.

ROBERT H. WHITE—The factors affecting the successful freezing of bull spermatozoa. Univ. of Tennessee, Knoxville.

Some Interesting Facts for the Dairy Farmer

Experience with price supports indicates that farmers must choose between actually cutting production sharply or accepting prices which move products into use. The question of how many farmers really desire to cut their own output is the key to the problem.

Farms are getting larger. (Illinois has 19,000 fewer farms now than in 1950, or a loss of 10%.) Main reasons for the drop include the economic advantages of large mechanized farms and the employment opportunities in other fields.

If a farmer reduces costs, he is likely in the long run to increase farm production unless the saved labor becomes leisure time, the money saved goes for household use, and the resources released move into nonagricultural uses.

Bulk milk tanks must be installed so as to meet certain requirements in most markets. A farmer should check with his local health department before building or remodeling a milk house for bulk handling.

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LETTERS TO THE EDITOR

Membership Responsibility

"Let's wake up and live!" says Mr. Judkins in the January 1956 issue of the Journal under the heading "Member Responsibility." I would like to modify this expression into: "Let's wake up and live the REALITY!" Members and nonmember dairy scientists, wake up and let your officers know what you want and what you don't want in your Dairy Science Association and in the Dairy Science Journal. An association and a journal can't be run successfully when it is carried only by a minority of its membership. The proof may be taken from Mr. Judkins' letter.

In his letter he complains that the Journal expenses threaten to outgrow the Journal income. Why? Mr. Judkins concludes: Not enough subscriptions to make the printing of the journal economical. I wonder whether this is the core of the problem? If in industry a certain product is lacking customers, the manufacturing will be rearranged until it will meet the demands of the customer. This rearrangement may affect quality and retail price. If rearrangment means concentration and improvement of parts of the product, it means also the elimination of nonessential parts. Such rearrangement will result in better marketing, favored by a lower retail price. A lowered price favors more consumption. Increased consumption may lower the costs of production per unit.

This law of economy may be translated into the conditions of the Journal of Dairy Science. A rearrangement would have to be done to develop a greater demand on the market! Further rearrangements would have to be done to concentrate on essential parts and to eliminate nonessential parts of the journal, in order to decrease costs and retail price. After reading Mr. Judkins' letter, it does not seem as if the management of the Dairy Science Journal is using to the fullest extent the knowledge of the law of "offer and demand." The journal management cannot expect that uneconomic considerations in the Journal's production and composition will be endorsed indefinitely by the members by means of increased subscription fees.

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Why couldn't an investigation be undertaken to find out the demands of the public on its Dairy Science Journal? The results of such an investigation would include among others the following results: Groups of "consumers," like scientific libraries, extension libraries, schools, research institutions, dairies, ice cream plants, cheese manufacturers, supply companies, artificial breeding stations, dairy farms, and individuals, will appear with preferences for certain parts of the Journal. There will be the pure researcher who has no interest in articles about applied dairy science; there will be the milk industry and food technology with no interest in research reports about the rumen digestion of a heifer or the feces collection of pasturing cattle; there will be the man from the artificial breeding station with no interest in articles of the two other groups. Despite these facts each has to buy the other's article because all are printed together in one volume. The actual value of the Journal is smaller for each "consumer" than the cost of the subscription. The businessman would probably answer the results of such investigation: "If one single product cannot meet everybody's desires and needs, why not make two or three part-products for the two or three different groups?"

Is a comparison with industry possible for the Journal of Dairy Science? Is a plan of a sectionized journal advisable? In great number the membership should respond to this vital question. There are definite economical advantages in specialization over generalization. On the other hand, does a sectionized Dairy Science Journal agree with the principle that we want to disseminate a universal understanding of Dairy Science beginning with the cow, her metabolism, her products, the interfering germs, to the butter, cheese, and ice cream manufacturing? We know, however, that the man with maximum accomplishments is usually strongly concentrated upon one item and negleets all other branches. It is the trend of modern industry and science to specialize in one field, to root deeply there and become an expert rather than scratching on the surface of a broad field and taking no deep roots at any place. The members of the Dairy Science Association will have to decide whether it is a good policy for the journal management to go with the time and its trend or whether it is better to keep conditions of times 50 years ago when all scientific journals were by far not as specialized as they are today.

GEORGE F. W. HAENLEIN
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A SURVEY OF VARIOUS FREE AMINO AND FATTY ACIDS IN DOMESTIC SWISS CHEESE[1]

P. C. HINTZ, W. L. SLATTER, AND W. J. HARPER
Department of Dairy Technology, Institute of Nutrition and Food Technology
The Ohio State University, Columbus

Limited research has been conducted on domestic Swiss cheese concerning the identification and measurement of the individual degradation products which result from the ripening process.

The workers who have studied the amino acids present in domestic Swiss cheese *(2, 11)* have analyzed relatively few samples of undetermined age. The limited results on domestic cheese appear to be in general agreement with the more extensive findings on Swiss and Gruyère cheese reported by European workers *(3, 21, 22)*. The amino acids which have been most frequently mentioned as being present in largest concentrations are glutamic acid, proline,valine, leucine, and serine. The amounts of several amino acids, especially proline and valine, have been considered to be associated with the sweetish component of the characteristic flavor of Swiss type cheese *(21, 22)*. Other investigations have emphasized that amino acids are not the only compounds associated with the flavor of Swiss type cheeses *(3, 22)*. The lower fatty acids, especially propionic and acetic acids, have been related to characteristic flavor *(3, 10, 12)*. Butyric acid, on the other hand, has been associated with poor flavored domestic Swiss cheese *(12)*.

Previous investigators have not simultaneously analyzed Swiss cheese for free fatty and amino acids. Therefore, in this survey analyses were made for representative free amino and fatty acids in commercial domestic Swiss cheese.

PROCEDURE

Samples of 16 commercially manufactured Swiss cheeses were obtained from six different factories of the Ohio Swiss Cheese Association. Although the cheeses were from different plants, the methods used in their manufacture were similar. All cheeses were made from raw milk with the same starter organisms. The starter consisted of *Streptococcus thermophilus* (4-6 ml/100 lb. milk), *Lactobacillus bulgaricus* (8-12 ml/100 lb.) and *Propionibacterium shermanii* (1 ml/100 lb.). The variations in manufacturing and curing practices are tabulated as follows: *(a)* forewarming, varied from 15-45 minutes, *(b)* cooking, 28-31 minutes, *(c)* "stirring out," varied from 14-45 minutes, *(d)* salting, in brine for 2 days at a temperature between 50° and 56° F., *(e)* warm room curing, cheese held 3 to 6 weeks at 70°-75° F., and *(f)* cold room curing, cheese held between 50° and 56° F.

Received for publication July 15, 1954.

[1] Scientific Paper 7-54. Department of Dairy Technology, The Ohio State University. Supported in part by the Ohio Dairy Products Research Fund.

The cheeses were selected to represent different grades and normal commercial manufacture. They varied in age from 1 to 11 months. Samples were judged organoleptically by a panel of three judges, and the cheeses were rated from 0 to 4 with respect to the intensity of characteristic Swiss cheese flavor; 0 designating no characteristic flavor intensity (CF-4).

Water extracts of 10-g. cheese samples were prepared by the method of Sommer and Harper *(19)* as modified by Long *(13)* for amino acid analysis. For immediate use, the 250-ml. aliquot of the 500-ml. extract was concentrated at low temperature (5° C.) under reduced pressure (20 mm.). When the concentrated extract could not be analyzed within 24 hours, it was lyophilized and stored at room temperature in a desiccator.

Tyrosine, phenylalanine, tryptophane, histidine, and lysine were separated on a 15-cm. long Dowex 50 column by the method of Moore and Stein *(15)*. As reported by these workers, tyrosine and phenylalanine did not separate completely. Therefore, the results for these two compounds are given as the sum of their individual concentrations.

The Moore and Stein *(15)* method as modified by Hamdy *et al. (6)* was used to measure cysteic acid, taurine, aspartic acid, threonine, serine, asparagine, glutamine, proline, and glycine. Since the method had not been shown to be usable for analysis of the leucines, valine, cystine, or methionine, these compounds were not included in this survey. The lot of Dowex 50 resin used for these experiments had a relative low porosity, which resulted in a flow rate of about one-half of that used by Hamdy *et al. (6)*. The resolution of threonine and serine, and glutamine and asparagine, was improved slightly. However, a relatively high concentration of any single compound generally resulted in a marked overlap of this amino compound with the related compound. Therefore, serine and threonine are reported together, as are asparagine and glutamine. The amount of each acid in the cheese was determined quantitatively by the method of Stein and Moore *(20)*.

An unknown acid, which was eluted between cysteic acid and taurine, was eluted from some cheese extracts. This study was completed before the compound was identified as serine-phosphate *(6)*. No effort was made to determine the amount of this acid present in the cheeses.

Acetic, propionic, butyric, valeric, and the higher fatty acids were measured by the direct chromatographic method of Harper *(9)*. Two and a half gram samples were used, and the results were reported as milligrams of acid per gram of cheese. Large concentrations of pyruvic acid frequently prevented quantitative measure of acetic acid because of a broad overlap of these two compounds in some cheeses. For the group of higher fatty acids the average molecular weight was assumed to be 228.

EXPERIMENTAL RESULTS

The age and intensity of characteristic flavor and the free acidic and neutral amino acid values of the 16 samples of cheese are presented in Table 1. The samples are arranged in order of increasing flavor intensity. In general, the

TABLE 1

Free acidic and neutral amino acids in commercial Swiss cheese

Sample No.	CF[a]	Age	Amount of amino acid per g. of cheese							
			Cysteic acid	Taurine	Aspartic acid	Threonine & serine	Asparagine & glutamine	Glutamic acid	Proline	Glycine
		(mo.)	*(mg.)*	*(mg.)*	*(mg.)*	*(mg.)*	*(mg.)*	*(mg.)*	*(mg.)*	*(mg.)*
1	0	1	0.73	0.70	2.60	1.90	0.80	0.87	0.30	0
2	0	1	0.60	0	0	2.00	2.30	0.20	0.10	0
3	0	1	0.60	0.02	4.00	0.47	0.47	1.10	0.70	0
4	0	4	1.10	0	0.68	1.20	0	0.25	0	0
5	0.5	3	0.46	0	0	5.70	0	1.90	2.00	2.40
6	0.5-1	2	0.63	0	0.71	—	—	1.00	0	0.57
7	1	3	0.75	0.13	(trace)	1.70	0.70	2.10	3.50	0.90
8	1	3	0.76	0.55	0	2.80	0	2.80	1.60	0.90
9	1-2	3	1.30	0	1.80	4.00	0	6.10	2.60	1.20
10	2	3	1.10	0.60	0	1.30	2.70	3.30	3.00	1.20
11	2-2.5	11	3.60	0	0.51	4.10	0	1.70	3.40	1.50
12	2-3	8	0.56	6.60	0	2.80	0.31	1.00	5.80	1.40
13	2.5-3	3	1.10	3.20	2.20	7.30	3.00	6.00	4.40	2.50
14	2.5-3	7	1.10	0.60	0	1.30	1.50	2.40	2.60	2.40
15	3	11	5.10	2.80	1.60	4.50	0	1.80	5.30	1.90
16	3.5-4	11	0.76	3.10	2.50	3.90	0	3.00	3.90	1.60

[a] Characteristic flavor: CF-0 = none; CF-1 = mild; CF-2 = medium; CF-3 = pronounced; CF-4 = very pronounced.

results are similar to those of previous investigators except that proline was found in amounts higher than glutamic acid in about half of the samples. The differences in the quantitative concentrations of these acids between different samples are greater than those previously reported, which may be accounted for by the differences in the methods of analysis, differences in raw milk microflora, and/or differences in the age of the samples studied. Quantitative analysis of proline by paper chromatography is very difficult *(13, 20)*.

In cheese less than 3 months of age, glutamic acid was present in relatively low concentrations, whereas proline and glycine were low in concentration or absent in these same samples. With the exception of Sample 4, these three acids were present in the aged cheeses in relatively large concentrations. Sample 4 was an exception to all general trends. Even at 4 months this cheese was completely lacking in characteristic Swiss cheese flavor and contained the lowest concentration of glutamic acid and no proline or glycine. The amount of aspartic acid varied from 0 to 4 mg., with no relationship to the age of the cheese.

The threonine-serine combination was found in all of the 15 samples that were analyzed for these acids. The asparagine-glutamine combination was present in some samples and absent in others.

Cysteic acid and taurine are compounds which have not been reported previously in Swiss cheese. In order to determine if cysteic acid was present as a result of the analytical procedure, cystine and cysteine were added to ground cheese just prior to extraction and concentration. Analysis of the concentrated extract revealed no increase in the cysteic acid content of the "cystine-cysteine enriched cheese" as compared to the original cheese sample. Cysteic acid was found in all of the samples, whereas taurine was found in 10 of the 16 samples.

Of the acidic and neutral amino acids, only glycine and proline appeared to be directly related to flavor development. In cheeses lacking characteristic flavor, glycine was not found and proline was absent or in low concentrations. Samples with a flavor rating of 2.5 or higher usually contained more than 2.5 mg. of proline. Exceptions to this trend were Samples 7 and 9, which contained more than 2.5 mg. of proline and were given a flavor rating below 2.5.

The free aromatic and basic amino acid content of the Swiss cheese samples is presented in Table 2. Lysine and the tyrosine-phenylalanine combination were present in all samples, whereas histidine was not found in four samples and tryptophane was missing in Sample 9. Three of the samples without histidine were the 1-month-old samples. No definite trends were observed in the concentrations of any of these amino acids and the age of the cheese.

The accumulation of free glutamic acid and proline was studied during the early stages of ripening of three different Swiss cheeses. Typical data are shown in Figure 1. It should be noted that after 15 days a decrease in glutamic acid is accompanied by a corresponding increase in proline.

Results of the analysis for fatty acids are shown in Table 3. The number corresponds to the cheeses in Tables 1 and 2, and the results are presented in order of the increasing flavor intensity of the cheese. Only cheeses 2 months old or older were analyzed. Butyric, propionic, and acetic acids were present in

TABLE 2

Free aromatic and basic amino acids in commercial Swiss cheese

Sample No.	CF[a]	Amount of amino acids per g. of cheese			
		Tyrosine and phenylalanine	Tryptophane	Histidine	Lysine
		(mg.)	*(mg.)*	*(mg.)*	*(mg.)*
1	0	1.90	0.72	0	2.30
2	0	7.90	1.00	0	1.60
3	0	4.60	0.87	0	2.50
4	0	1.10	0.98	2.50	0.22
5	0.5	1.60	3.00	0.65	0.70
6	0.5-1	3.70	5.50	0.74	3.00
7	1	3.90	(trace)	3.50	1.50
8	1	4.40	0.08	0.11	0.53
9	1-2	1.70	0	0.53	1.70
10	2	5.40	0.54	1.10	1.00
11	2-2.5	4.60	1.50	0.40	4.80
12	2-3	4.20	0.08	0.30	2.80
13	2.5-3	3.60	0.82	0.36	—
14	2.5-3	1.50	1.20	0.46	1.90
15	3	2.90	0.64	0	9.50
16	3.5-4	1.70	2.20	3.70	2.20

[a] Characteristic flavor: CF-0 = none; CF-1 = mild; CF-2 = medium; CF-3 = pronounced; CF-4 = very pronounced.

concentrations similar to those reported by other investigators. The concentrations of butyric acid, ranging from 0.32 to 1.6 mg. per gram, did not appear to be related to the age of the cheese. Propionic acid contents varied from 3.1 to 6.2 mg. per gram with three samples containing more than 5.5 mg. In the samples that could be analyzed for acetic acid, the concentrations ranged from 1.8 to 3.9 mg. The product interfering with separation of acetic acid was identified as pyruvic acid by its R_f value and by its 2,4-dinitrophenylhydrazine derivative.

TABLE 3

Various free fatty acids in commercial Swiss cheese

Sample No.[b]	CF	Age	Amount of fatty acids per g. of cheese				
			Higher acids[a]	Valeric acid	Butyric acid	Propionic acid	Acetic acid
		(mo.)	*(mg.)*	*(mg.)*	*(mg.)*	*(mg.)*	*(mg.)*
4	0	4	5.0	0.32	1.6	3.1	1.8
5	0.5	3	2.3[c]	—	1.5	4.3	2.4
6	0.5-1	2	5.0	0.84	1.2	4.3	+[d]
7	1	3	3.0[c]	—	0.72	4.3	+[d]
8	1	3	2.4[c]	—	1.2	4.4	2.6
9	1-2	3	5.9[c]	—	1.1	5.9	+[d]
10	2	3	2.0[c]	—	0.32	5.1	3.1
11	2-2.5	11	4.7	0.18	0.53	4.6	+[d]
12	2-3	8	3.9	0.37	0.64	6.2	3.9
13	2.5-3	3	3.4[c]	—	0.58	5.6	2.6
14	2.5-3	7	10.6	0.48	0.58	5.0	+[d]
15	3	11	24.2	0.19	1.1	5.0	3.0
16	3.5-4	11	12.5	0	0.77	5.5	+[d]

[a] C_6 and above; average molecular weight of 228 assumed.
[b] Analyses not made on Samples 1, 2, and 3.
[c] Includes valeric acid.
[d] Acid present but pyruvic acid interference prevented a quantitative measure of the acid.

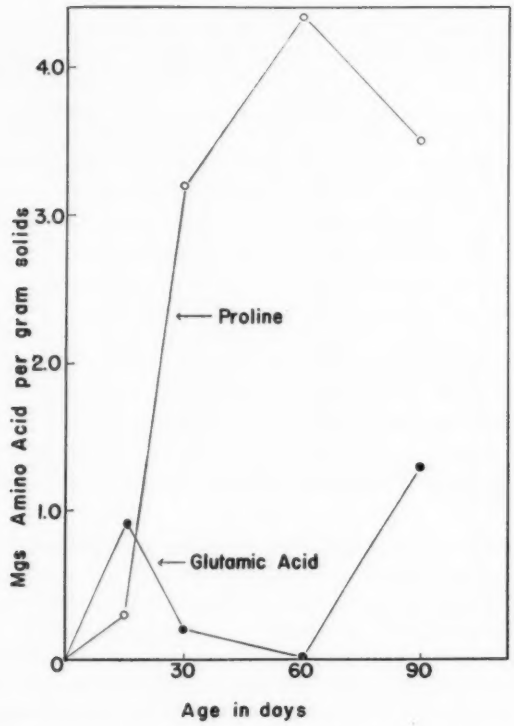

FIG. 1. Accumulation of free glutamic acid and proline during the early stages of ripening of Swiss cheese.

The concentrations of propionic and acetic acids were not related to the age of the cheese, indicating that these acids may be produced rather rapidly with only slow increases occurring after the cheese is 3 months old.

Most good-flavored cheeses had at least 5.0 mg. of propionic acid per gram, whereas those receiving flavor ratings of less than 2 contained less than this amount.

A high concentration of butyric acid was noted in the samples that possessed undesirable flavor (Samples 4 and 5), which corroborates the findings of Krett and Stine *(12)*. The concentration of higher fatty acids (above C_5) varied from 2.0 to 24.2 mg. per gram of cheese solids and showed no relationship with the age of the cheese or with the concentrations of other acids.

The presence of valeric acid is noteworthy, since this acid has not been reported previously as being present in Swiss cheese. Its presence has been reported in Stilton and Roquefort cheese *(10)*. It was found in all but one of the seven cheeses analyzed for this acid. It varied in concentration and could not be related to age or flavor of the cheese or to the concentration of other

TABLE 4

Flavor relationship between proline and propionic acid[a]

CF 0-0.5[b]	CF 0.5-1.0		CF 1-2		CF 2+	
Proline	Proline	Propionic	Proline	Propionic	Proline	Propionic
(mg.)	*(mg.)*	*(mg.)*	*(mg.)*	*(mg.)*	*(mg.)*	*(mg.)*
0.3	0	4.3	2.6	5.9	3.4	4.6
0.1	2.0	4.3	2.0	5.1	5.8	6.2
0.7	3.5	4.3			4.4	5.6
0	1.6	4.4			2.6	5.0
					5.3	5.0
Average					3.9	5.5
0.3	1.8	4.3	2.3	5.5	4.2	5.3

[a] Concentrations presented in mg. per g. of cheese.
[b] Propionic acid was not measured for these cheeses.

fatty acids. It is interesting to note that α-amino-valeric acid has been found in Herve (Limburger type) cheese but never in Gruyère *(18)*.

The results obtained (Tables 1 and 3) indicated a general interrelationship between proline, propionic acid, and flavor intensity. To emphasize this, direct comparisons are made in Table 4 between the concentrations of these acids and flavor ratings. Generally, the high flavor ratings appear to be associated with the presence of minimum amounts of both proline and propionic acids. For example, one cheese (Sample 7) with low flavor rating contained 3.5 mg. of proline per gram and only 4.3 mg. of propionic per gram. In another sample (No. 5), a cheese receiving a low flavor rating contained 4.3 mg. of propionic acid per gram of cheese and no proline.

DISCUSSION

This investigation revealed that considerable variation occurs in the free amino acid content of various domestic Swiss cheeses. The variation in the milk supply and in cheese manufacturing may be related to these variations. The results of these studies are not always in agreement with those of Block *(2)*, Storgards and Lindquist *(21)*, and Kosikowsky *(11)*. Interpretation of these differences is not possible because of differences in methods of analysis, age of cheese, and manufacturing conditions. The results reveal that in most cheeses in which the proline content was high, the concentration of glutamic acid was relatively low as compared to the values reported by other workers. Fruton and Simmonds *(5)* have reported that glutamic acid is a precursor of both ornithine and proline in microbial metabolism. Since the concentration of glutamic acid in Swiss cheese is lower than in either Italian or Cheddar types *(9, 13)*, the assumption might be made that in Swiss cheese the glutamic acid has been converted to proline or is formed in lesser amounts. However, the limited study of the amino acid content of Swiss cheese during early stages of ripening tends to substantiate the former view.

Cysteic acid, which was present in all the cheeses, might be assumed to be an oxidation product of cysteine, since it is not found in the casein molecule itself and since taurine is also found in some cheeses. However, in several instances there was more cysteic acid present than could be accounted for by

the total cystine-cysteine content of the casein in the cheese. Therefore, the exact mechanism of cysteic acid formation remains to be clarified. Addition of cysteine or cystine to cheese extracts prior to extraction did not increase either the cysteic acid or taurine values. Nor did addition of cysteic acid prior to extraction result in increased taurine. The taurine would presumedly result from the decarboxylation of cysteic acid.

$$\text{Cysteine} \longrightarrow \text{Cysteic acid} \xrightarrow{-CO_2} \text{Taurine}$$

The experimental evidence in this study supports the suggestion of others that free proline is associated with flavor *(3, 22)*.

Two samples of Swiss cheese having excessively high concentrations of histidine had low flavor ratings. Long and Harper *(14)* have reported that histidine was undesirable in other cheeses, mainly Cheddar and Provolone. However, the cheese having the highest flavor rating in this study also had the highest concentration of histidine, which may indicate that histidine does not always exert a deleterious effect.

The data support those of Krett and Stine *(12)* that high butyric acid content is detrimental to good Swiss cheese flavor; also, that high propionic acid and acetic content accompanies a good flavor.

The evidence indicates the possibility of the need for certain concentrations of propionic acid and free proline if the flavor of the cheese is to be good.

SUMMARY

This investigation was undertaken to identify and measure some of the free amino and free fatty acids in commercial Swiss cheese of varying ages and to study their relation to flavor. Sixteen samples of cheese varying in age from 2 to 11 months were used in the study.

The cheeses were analyzed by means of a polystyrene ion-exchange method for the following free amino acids: cysteic acid, taurine, aspartic acid, threonine, serine, asparagine, glutamic acid, proline, glycine, tyrosine, phenylalanine, tryptophane, histidine, and lysine. Determinations were made by buffered silica gel chromatography of the following free fatty acids or groups: valeric, butyric, propionic, and fatty acids of the C_6 and above.

Not all of the free amino acids were always present in the cheeses, and there was a wide variation among the samples in concentration of the different acids. Only cysteic acid, threonine-serine, glutamic acid, and tyrosine-phenylalanine were present in all samples. Proline showed the widest variation in content of all the amino acids measured, ranging from zero to 5.8 mg. per gram of cheese.

Average amounts of cysteic acid, taurine, proline, lysine, and histidine tended to increase with the age of the cheese, whereas the amount of glycine remained relatively constant when present. The amounts of aspartic acid, threonine-serine, asparagine-glutamine, glutamic acid, tyrosine-phenylalanine, and tryptophane could not be related to the age of the cheese.

Butyric, propionic, and higher acids were present in all samples studied, and valeric acid was present in all but one of the samples. There was no relationship between age and the amounts present.

Cysteic acid, taurine, and valeric acid, which were not previously reported as being present in Swiss cheese, were found in some samples of cheese.

An interrelationship between proline and propionic acid content and flavor was found. A minimum proline concentration of 2.0 mg. per gram and a minimum propionic acid concentration of 5.0 mg. per gram of cheese were present before the cheese possessed a satisfactory Swiss cheese flavor. Values of either of these two acids below the minimum were associated with low flavor ratings.

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ITALIAN CHEESE RIPENING.
VI. EFFECTS OF DIFFERENT TYPES OF LIPOLYTIC ENZYME PREPARATIONS ON THE ACCUMULATION OF VARIOUS FREE FATTY AND FREE AMINO ACIDS AND THE DEVELOPMENT OF FLAVOR IN PROVOLONE AND ROMANO CHEESE[1,2]

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Previous papers in this series have dealt with the concentrations of various free amino and fatty acids in commercial Provolone and Romano cheese *(3, 4)*. Fat degradation, specifically the production of free butyric acid, appeared to be directly related to the type of enzyme product used in the manufacture of cheese *(2, 3, 4)*. In contrast, the free amino acid content was not related to the enzyme products used but to the action of the bacterial flora *(3)*.

However, the significance of these findings could not be evaluated, since the cheeses were made in various factories under different conditions. Therefore, this study was made in order to evaluate the effects of different types of lipolytic enzyme products on the ripening of Italian cheese made in one factory under controlled conditions.

PROCEDURE

Four vats of Romano and four vats of Provolone cheese were manufactured from the same milk supply and the same starter culture *(Lactobacillus bulgaricus)*. The only variable introduced was the type of enzyme preparation used for each of the four cheeses. For both Romano and Provolone, one lot each was made with the following: *(a)* rennet extract alone (Hansen), *(b)* rennet extract (Hansen) plus calf glandular preparation (Italase), *(c)* rennet extract plus kid glandular preparation (Capalase), and *(d)* imported crude kid rennet paste.

Each variety of cheese was manufactured by a commercially recognized method and in a commercial cheese factory. The manufacturing methods for each lot of each specific variety were maintained constant, so that the temperatures and vat acidities were identical for all lots at any specific stage of the manufacturing process. The manufacturing details are shown in Tables 1 and 2 for Provolone and Romano, respectively. The Provolone cheese was smoked and

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TABLE 1

Manufacturing data for a typical Provolone cheese

Amount of milk	200 lb.
Fat test of milk	3.3%
Acidity of milk	0.19%
Acidity of starter	1.3%
Amount of starter	1.5% *(Lactobacillus bulgaricus)*
Amount of rennet	3 oz/1,000 lb. of milk
Amount of lipase	4 oz/1,000 lb. of milk
Temperature at setting	90° F.
Time to set	27 min.
Total time to cook	30 min.
Temperature of cooking	117° F.
Time from setting to draining	65 min.
Acidity at draining	0.16%
Time from setting to mixing	150 min.
Temperature of mixing water	165° F.
Temperature and time of mixing curd	Average of 140° F. for 25 min.
pH at mixing	5.3
Salting	Brine salted

TABLE 2

Manufacturing data for a typical Romano cheese

Amount of milk	200 lb.
Fat test of milk	2.5%
Acidity of milk	0.185%
Acidity of starter	1.3%
Amount of starter	1.5% *(Lactobacillus bulgaricus)*
Amount of rennet	3 oz/1,000 lb. of milk
Amount of lipase	4 oz/1,000 lb. of milk
Time from setting to cut	25 min.
Temperature of cooking	115° F.
Total time to cook	30 min.
Time from setting to draining (1st drain)	60 min.
Time from setting to draining (2nd drain)	90 min.
Time from setting to dipping curd	100 min.
pH at dipping	6.1 (0.23% acidity)
pH out of forms	5.3
Salting	Brine one day and then dry salted

brine-salted and the Romano cheese was brine-salted before being transferred to The Ohio State University. The dry salting of the Romano was completed at the University. The cheeses were stored for 1 year at a temperature of 50° ± 2° F. and a relative humidity of 75%.

The sampling techniques and methods for determining moisture and water-soluble nitrogen were the same as those used in previous work *(3, 4)*. Analyses for free amino acids and fatty acids were made at 1, 14, 30, 60, 90, 220, and 360 days of age. Analyses were made for free alanine, aspartic acid, glutamic acid, glycine, histidine, the combined leucines, methionine, threonine, and valine; and for free acetic, propionic, butyric, and a group of "higher" fatty acids. The free amino acids were measured by the paper chromatographic method, and the free fatty acids were measured by the direct chromatographic method previously described *(1, 3)*.

RESULTS

The results of analyses at various times of ripening are similar for Provolone and Romano cheese. For convenience the results for each cheese variety are presented separately.

Provolone cheese. The intensities of the characteristic flavor of the four Provolone cheeses during the ripening period are shown in Table 3. The cheese made with rennet extract exhibited the least flavor development, whereas the cheese made with the kid glandular preparation and the kid rennet paste showed the greatest and most rapid development of flavor.

TABLE 3

Development of characteristic flavor in Provolone cheese made with different enzyme preparations during 360 days of ripening at 50° F.

Sample No.	Source and type of enzyme[b]	Characteristic flavor score[a]					
		Age (days)					
		15	30	60	90	220	360
50	Rennet extract	0	0	0	0	0	0
51	Calf gland enzyme	0	0	0	0.5	2	2
52	Kid gland enzyme	0	?	0.5	1.5	3	3.5
53	Kid rennet paste	0	?	0.5	1	2	3.5

[a] Characteristic flavor: intensity from 0 to 4.
[b] Rennet extract also used with all glandular preparations.

The four different treatments of milk with coagulating and ripening enzymes produced practically the same development of propionic and acetic acids during the one year of curing. The propionic acid was detectable, but not measurable, at 1 day of age; it increased from approximately 0.10 mg. per gram of cheese solids at 14 days to 0.23 mg. at 370 days. Acetic acid in milligrams per gram of cheese solids at 1, 14, 30, 60, 90, 220, and 370 days of curing approximated 0.05, 0.15, 0.25, 0.32, 0.23, 0.32, and 0.40, respectively.

TABLE 4

Butyric acid present in Provolone cheese made with various enzyme preparations

Age at analysis	Butyric acid[a] in Provolone made with:			
	Rennet extract	Rennet plus calf glandular preparation	Rennet plus kid glandular preparation	Kid rennet paste
1	0.05	0.12	0.13	0.13
14	0.31	0.39	0.55	0.66
30	0.51	0.88	1.20	1.50
60	0.55	1.10	1.60	1.80
90	0.55	1.30	1.80	2.20
220	0.55	1.50	2.30	2.60
370	0.71	1.70	2.90	3.20

[a] Mg. acid per gram of cheese.

The free butyric content measured in the cheese at 1, 14, 30, 60, 90, 270, and 370 days is given in Table 4. As previously reported for cheese manufactured under varied conditions *(3)*, a definite relationship was found to exist between the kind of enzyme product used in the manufacture of the cheese and the development of free butyric acid in the cheese. Of the free fatty acids, only butyric could be related to the enzyme product. Even at 14 days this relationship was apparent, and at 60 days the concentration of the free butyric acid was directly related to the type of enzyme product, which in turn was related to the flavor

development of the cheese. The cheese made with rennet extract showed little increase in free butyric acid after the first 30 days, whereas the cheese made with enzyme preparation containing active lipase showed a gradual increase of free butyric acid throughout the ripening period.

At 1 day of age free alanine, glutamic acid, leucines, and valine were detectable, but not present in measurable concentrations. As the cheese aged, the free leucines were present in the greatest concentrations, followed by the glutamic acid, valine, and alanine. Glycine and threonine were not detected until after 60 days of storage, whereas methionine and histidine were present in some and absent from other samples at 60 days. The free amino acid values were not identical in the four different samples, but the values could not be related to the kind of enzyme product used in the manufacture.

The greatest changes in concentration occurred after 90 days of storage. A comparison of the free amino acid content at 90 and 370 days is given in Table 5. There was generally a marked increase in almost all amino acids between 90 and

TABLE 5

Concentrations of various free amino acids liberated at 90 and 370 days in the ripening of Provolone cheese made with various enzyme preparations

Treatment:	Rennet extract		Rennet plus calf glandular preparation		Rennet plus kid glandular preparation		Kid rennet paste	
Age in days:	90	370	90	370	90	370	90	370
Amino acids	*(mg.)*	*(mg.)*	*(mg.)*	*(mg.)*	*(mg.)*	*(mg.)*	*(mg.)*	*(mg.)*
Alanine	1.10	2.20	1.80	2.60	1.30	1.90	1.40	2.30
Aspartic acid	0.80	1.80	1.10	2.20	1.10	1.60	+	2.40
Glycine	+[a]	1.30	0	1.10	+	1.40	+	1.10
Glutamic acid	1.60	5.80	1.90	6.10	2.00	5.60	2.10	6.30
Histidine	0	0.70	0	0	0	0.40	0	0.30
Leucine	3.40	5.90	3.70	7.20	3.80	6.80	3.20	7.60
Methionine	0.30	0.30	+	0	0.20	0.50	0.30	0.30
Threonine	0	0.20	0	0.20	0	0.10	0	0.20
Valine	1.50	2.10	0.70	1.70	1.30	2.00	1.40	2.30

[a] + indicates less than 0.1 mg/g cheese solids.

370 days. The free amino acids were present in relatively large quantities in the year-old cheese, with the leucines and glutamic acid being present in the highest concentrations.

One interesting observation was that as the cheese aged, the variation in the free amino acid content of the various cheeses became less. In the year-old cheese the amino acid contents were similar, with the exception of histidine and methionine. These acids were present in some cheeses and absent in others.

Romano cheese. The characteristic flavor scores of the four Romano cheeses are given in Table 6. As in Provolone, the cheeses made with rennet extract showed very little characteristic flavor development during the ripening period. The cheese made with kid enzyme products showed the greatest intensity of flavor development.

The degradation of fat was definite at 1 day, and all of the lower fatty acids were present in measurable amounts. As in Provolone cheese, the four different

TABLE 6

Characteristic flavor intensity of experimental Romano cheese during 370 days of ripening[a]

Sample No.	Source and type of enzyme	Characteristic flavor intensity					
		Age (days)					
		15	30	60	90	220	370
1	Rennet extract	0	0	0	0	1	1
2	Calf gland enzyme	0	0	0.5	0.5	3	3
3	Kid gland enzyme	0	0	0.5	1	3	4
4	Kid rennet paste	0	0.5	1	2	2.5	4

[a] Characteristic flavor; intensity from 0 to 4.

treatments of the milk with coagulating and ripening enzymes produced practically the same development of propionic and acetic acids during 1 year of curing. Propionic acid in milligrams per gram of cheese solids at 1, 14, 30, 60, 90, 220, and 370 days of curing approximated 0.1, 0.12, 0.17, 0.23, 0.03, 0.36, and 0.40, respectively. Acetic acid at 1, 14, 30, 60, 90, 220, and 370 days of curing approximated 0.1, 0.3, 0.4, 0.52, 0.56, 0.58, and 0.63 mg. per gram of cheese solids, respectively.

TABLE 7

Butyric acid present in Romano cheese made with various enzyme preparations

Age at analysis	Butyric acid[a] in Romano made with			
	Rennet extract	Rennet plus calf glandular preparation	Rennet plus kid glandular preparation	Kid rennet paste
1	0.15	0.30	0.45	0.51
14	0.29	0.52	0.72	1.10
30	0.53	1.20	1.40	1.80
60	0.62	1.80	2.20	2.60
90	0.72	1.90	3.00	3.40
220	0.90	2.40	3.90	4.20
370	1.20	3.20	5.50	5.70

[a] Mg. acid per gram cheese solids.

The amount of free butyric acid in the cheese at 1, 14, 30, 60, 90, 220, and 370 days is shown in Table 7. The results are similar to those previously shown for Provolone cheese, except that the compounds are present in much higher concentrations in Romano cheese. The relationship between lipase enzyme and characteristic flavor was observed and is apparent even in 1-day-old cheese.

Most of the free amino acids studied were present in the 1-day-old cheese, and only glycine and threonine were not detected. The greatest increase in free amino acid content occurred between 90 and 370 days of age.

The free amino and fatty acid content of the cheese at 90 and 370 days is shown in Table 8. The results are similar to those previously reported for Provolone, except that the compounds are present in much higher concentrations. Although there were differences in the amounts of various free amino acids, none of the variations could be related to the type of enzyme products used in the manufacture. The general pattern of free amino acid is the same as for Provolone.

TABLE 8

Concentrations of various free amino acids liberated at 90 and 370 days in the ripening of Romano cheese made with various enzyme preparations[a, b]

Treatment:	Rennet extract		Rennet plus calf glandular preparation		Rennet plus kid glandular preparation		Kid rennet paste	
Age in days:	90	370	90	370	90	370	90	370
Amino acids	*(mg.)*	*(mg.)*	*(mg.)*	*(mg.)*	*(mg.)*	*(mg.)*	*(mg.)*	*(mg.)*
Alanine	2.00	3.00	2.50	2.80	1.90	2.60	2.20	2.40
Aspartic acid	2.40	3.80	2.30	3.00	1.90	3.40	2.90	3.00
Glycine	0	1.70	+	1.80	0	2.10	0	1.90
Glutamic acid	3.60	7.10	2.80	6.90	2.40	8.30	5.50	7.20
Histidine	0	0.70	0	0	0	0	0	+
Leucine	4.30	8.00	5.70	8.30	5.60	9.10	5.30	8.40
Methionine	+	0.40	+	0.60	+	0	+	0.10
Threonine	0	0.40	0	0.30	0	0	0	0.10
Valine	1.30	2.90	1.70	3.20	1.60	3.60	1.50	2.90

[a] Mg. acid per gram cheese solids.
[b] + indicates less than 0.1 mg. amino acid present per gram.

DISCUSSION

This study, conducted under carefully observed conditions, basically substantiates the earlier observations pertaining to the relationship of the enzyme product to the ripening of Italian cheese. Rennet extract, which contained no active lipase, did not result in characteristic flavor development. The liberation and concentration of butyric acid in this cheese is similar to that reported for Cheddar cheese *(6)*. The two kid preparations resulted in cheese with similar flavor and similar butyric acid contents, whereas the calf rennet paste produced more butyric acid than the rennet extract, but not as much as the kid product. The confirmation of the proposed relationship between enzyme product, flavor production, and butyric acid formation is considered good evidence for the importance of the enzyme product in the ripening of Italian cheese. The results also show that the hygienically unsatisfactory rennet paste is not essential, because the desired characteristic flavor developed and butyric acid was formed in the cheese made with purified enzyme preparations from glandular sources.

The lack of relationship noted between the free amino acid content of the cheese and the enzyme product is further evidence that a second and independent factor is involved in the ripening of both Romano and Provolone cheese. Although both the Romano and the Provolone were made from the same lot of raw milk, all of the Provolone samples were phosphatase-negative as a result of the high temperature received during the molding process. This would account for the lower rate of degradation of both fat and protein in the Provolone cheese. It might also account for the greater uniformity in the free amino acid content of Provolone cheese as compared to that of Romano cheese.

The concentration of many of the free amino acids showed tendencies to first increase, then decrease, and subsequently again increase in concentration as the ripening period progressed. Such changes in end-product concentration could not be related to the lipase enzyme preparations and, therefore, these changes probably reflect changes in the bacterial flora during ripening and in the utiliza-

tion of certain amino acids in the metabolism of microorganisms. Thus, the compounds available at any given time would tend to direct the growth of different types of flora, depending on the enzyme system of the microorganisms. Therefore, to insure the accumulation of the desired end products and proper flavor productions, attention must also be given to those enzyme systems which enable the bacteria to utilize certain amino compounds as sources of carbon and energy. The differences in raw and pasteurized milk cheese may be explained not only by differences in active proteolysis but partially by the subsequent utilization of certain amino compounds by microorganisms or by chemical reaction with other compounds which provide the desired end result.

The presence of certain amino acids, such as glutamic acid and the leucines, in high concentrations in the 370-day-old cheese undoubtedly has an influence on the flavor characteristics of the cheese. However, the absence of any particular "free" amino acid in the cheese should not be considered as evidence that the compound is not liberated or is not important. Rather, this absence might only indicate the presence of an active enzyme system capable of converting the compound into a secondary compound, which in turn could be a part of the flavor. Therefore, attention might be given also to compounds that may be present in relatively low concentration during ripening, as related to their concentration in casein. Threonine, methionine, and histidine are notable examples of compounds present in low concentrations. These compounds have been shown to play an important part in bacterial metabolism, and their transformation may be important in the development of cheese flavor.

SUMMARY

Each of four vat lots of milk was treated with one of four different enzyme combinations and made into Provolone cheese. Another series of four vat lots of milk was treated in the same manner and made into Romano cheese. Various free amino and free fatty acids developed in the ripening cheese were measured chromatographically over a period of 1 year.

The rate of formation and the concentration of free butyric acid were found to be directly related to the type of enzyme product used in the manufacture of the cheese. The kid rennet paste and kid glandular lipase preparations resulted in cheese with the highest butyric acid content, the calf product was next, and the rennet extract resulted in a cheese with a very slow rate of butyric acid flavor production.

The enzyme product was not related to the accumulation of the free amino acids. A hypothesis of interconversion of amino compounds is discussed. The appearance of the desired cheese flavor was related to butyric acid and glutamic acid in Provolone cheese and to butyric acid in Romano cheese. Glutamic acid could not be related to the characteristic flavor of Romano. The leucines increased to a consistently higher level than did the glutamic acid in the ripening of Provolone cheese. Further work is in progress to investigate the significance of this finding.

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THE NEUTRAL CARBONYL COMPOUNDS IN BLUE-MOLD TYPE CHEESE

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The importance of methyl ketones as flavor constituents of cheese in which *Penicillium roqueforti* is the ripening agent was indicated by Starkle *(9)* and Hammer and Bryant *(2)* and more recently has been conclusively established by Patton *(6)*. The latter isolated pentanone-2, heptanone-2, and nonanone-2 by fractional distillation of the steam volatiles obtained from a rather large quantity of a domestic Blue cheese of good quality. Further investigation by this author indicates that heptanone-2 has a flavor and aroma typical of Blue cheese and that Blue cheese flavor could be simulated in salad oil by addition of this ketone and butyric acid *(7)*.

In light of the above reports the development of a simple and rapid test for qualitative and semi-quantitative evaluation of the ketonic flavor constituents of blue-mold cheese would seem desirable. Experience with a chromatographic procedure for the separation and identification of 2,4-dinitrophenylhydrazones of aldehydes and ketones *(5)* has led to the development of such a test.

EXPERIMENTAL PROCEDURE

Ten ½-lb. samples of blue-mold type cheese representative of the cheese sold at nearby retail outlets were obtained. No information concerning these samples other than the variety, brand, and source was available. The samples were examined for flavor, aroma, and mold growth.

Distillation of cheese samples. Fifty grams of cheese from which all traces of rind and outer portions had been removed was mixed in a Waring blendor with 50 ml. of water until homogeneous. The cheese suspension was poured into a 250-ml. Erlenmeyer flask containing several glass beads and one drop of Dow Corning Silicone Antifoam A. Thirty ml. of 0.2% 2,4-dinitrophenylhydrazine in 2 *N* HCl was added to a 125-ml. Erlenmeyer flask. The two flasks were connected to a Y-shaped distillation tube similar to that described by Van Slyke *et al. (11)* by means of tight-fitting rubber stoppers. The third arm of the distillation tube was fitted with a short piece of heavy-walled rubber tubing and a screw-clamp. The assembled apparatus was connected to a mechanical vacuum pump protected by a calcium sulfate moisture trap. The distilling apparatus was swirled rapidly and evacuated until a head of foam developed on the cheese mixture. The vacuum was interrupted by closing the screw-clamp, and the cheese mixture was agitated until the foam dissipated. This procedure was repeated until the cheese mixture boiled gently without appreciable foam formation when the screw-clamp was opened momentarily. The flask containing the 2,4-dinitrophenylhydrazine solution was then immersed in an ice-water bath,

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thereby making the cheese mixture boil. Precipitation of derivatives of the carbonyl compounds usually began at this point. After this further evacuation of the cheese mixture was complete, the cheese flask was gradually immersed in a water bath maintained at 90° C. with continued swirling of the cheese mixture. When approximately 30 ml. of distillate had been collected in the cooled receiver, the vacuum was released and the receiver flask was stoppered. The distillate and 2,4-dinitrophenylhydrazine mixture was allowed to stand at room temperature for 1 hour with occasional shaking. During this time a heavy precipitate of 2,4-dinitrophenylhydrazones had formed.

Preparation and chromatography of carbonyl derivatives. The cheese distillate containing the mixture of carbonyl derivatives was extracted with 50-ml. and 25-ml. portions of 1:1 benzene-heptane. The solvent was evaporated quickly over a hot water bath in a stream of air. The dry residue was taken up in a minimum of warm benzene-heptane solvent and poured onto a 13×50 mm. column of anhydrous magnesium sulfate. When all of the solvent solution had flowed into the column, the 2,4-dinitrophenylhydrazones were eluted with fresh solvent under 3-5 lb. air pressure until the effluent became colorless. The unreacted 2,4-dinitrophenylhydrazine remained at the top of the column. The effluent was evaporated to a convenient volume and made to 10 ml. with solvent.

A 0.01-ml. quantity of the mixed derivative extract and appropriate quantities of benzene-heptane solutions of authentic 2,4-dinitrophenylhydrazones of acetaldehyde, acetone, butanone-2, pentanone-2, heptanone-2, nonanone-2, and undecanone-2 were spotted on Whatman No. 1 paper and chromatographed by the procedure of Heulin *(3)* as described in reference *5*.

Chromatographic fractionation of carbonyl derivatives. The derivative extract was streaked on $18\frac{1}{4} \times 11\frac{1}{2}$ in. sheets of Whatman No. 1 paper in an intense narrow band $1\frac{1}{2}$ in. from the long edge of the paper. This was accomplished by tying the sheet around the drum (diam. $6\frac{1}{4}$ in.) of a kymograph so that the line on which the derivative was applied was parallel to and about $\frac{1}{2}$ in. above the top end of the drum. The derivative solution was applied with a graduated 1-ml. capillary-tipped pipette, which was clamped in a horizontal position on the rod of a support stand. The drum of the kymograph was revolved at 6-7 r.p.m. and the pipette tip was brought into contact with the paper so that the derivative solution was drawn out along the origin line on the paper. A stream of air was blown on the paper near the pipette tip to effect rapid evaporation of the solvent and restrict the derivative band width to 3-4 mm. Depending on the concentration of derivative in the extract from various cheese distillates, from 0.2 to 1.0 ml. of extract was applied per sheet to assure adequate separation of the component derivatives.

From five to seven such papers were prepared for each sample, the papers cut in half at $9\frac{1}{2}$ in., formed into cylinders, and developed as described previously *(5)*. After development, the yellow bands were cut from the chromatograms and the separated carbonyl derivatives were eluted with methanol.

The derivative fractions which had been well separated on the chromatograms were recrystallized once or twice from ethanol. Melting points of the

isolated derivatives were made on a micro-hot stage. The three fastest moving fractions that were not completely separated by the first fractionation were subjected to a second fractionation in an attempt to remove traces of adjacent fractions. The identity and purity of fractions thus isolated were determined by chromatographing spots of these fractions along with known methyl ketone derivatives. Micro-melting point determinations were made where sufficient derivative had been isolated to permit recrystallization.

The known carbonyl 2,4-dinitrophenylhydrazones used in the chromatographic identification of the cheese carbonyl derivatives and for melting point determination were prepared by customary procedures *(8)*. One exception was the preparation of undecanone-2 derivative, in which all attempts to obtain a crystalline derivative were unsuccessful. This derivative was prepared by shaking undecanone-2 with an excess of 2,4-dinitrophenylhydrazine in 2 *N* HCl, extracting with benzene-heptane, and removing the excess reagent by adsorption on anhydrous magnesium sulfate. The resulting derivative solution was used for the chromatographic identification of undecanone-2 derivative obtained from cheese samples.

RESULTS

The aroma and flavor judgments on the ten different cheese samples examined for carbonyl compounds are given in Table 1. Samples 1 and 3 were criticized as possessing an aroma that was not characteristic of good Blue cheese, whereas the aroma of Sample 8 possessed little or no ketonic character. The flavor of samples varied from good to poor with Sample 4 being the only one not criticized.

TABLE 1

Description of cheese samples examined for neutral carbonyl compounds

Sample No.	Type	Aroma[a]	Flavor
1	French Roquefort	Atypical	Poor, sweetish, earthy
2	French Roquefort	Typical	Fair, slightly unclean
3	Danish Blue	Atypical	Fair, earthy
4	Danish Blue	Typical	Good
5	Domestic Blue	Typical	Fair, sweetish
6	Gorgonzola	Typical	Fair, slightly unclean
7	Domestic Blue	Typical	Poor, unclean
8	Domestic Blue	Atypical	Poor, cooked, lacks flavor
9	Domestic Blue	Typical	Poor, Cheddar-like
10	Danish Blue	Typical	Fair, slightly soapy

[a] Judged as to the presence or absence of desirable ketonic aroma.

Figure 1 is a tracing of a chromatogram of the 2,4-dinitrophenylhydrazones of the carbonyl compounds formed in the distillates from the ten cheese samples run with a mixture of derivatives of known carbonyl compounds. The chromatogram reveals a marked variation in the quality and quantity of carbonyl compounds obtained from the various cheeses. As was expected from Patton's work *(6)*, most of the samples yielded carbonyl compounds, the derivatives of which were chromatographically identical with derivatives of acetone, penta-

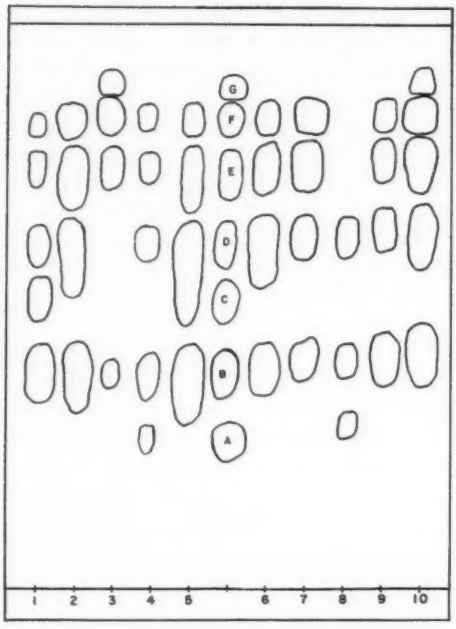

FIG. 1. The 2,4-dinitrophenylhydrazones of carbonyl compounds distilled from ten different samples of Blue-mold cheese chromatographed with derivatives of (A) acetaldehyde, (B) acetone, (C) butanone-2, (D) pentanone-2, (E) heptanone-2, (F) nonanone-2, and (G) undecanone-2.

none-2, heptanone-2, and nonanone-2. In addition, Samples 4 and 8 gave derivative spots corresponding to that for acetaldehyde derivative, and Samples 3 and 10 gave faint spots that ran with the spot of undecanone-2 derivative. Sample 1 gave a spot that ran parallel with the spot of the derivative of the even-numbered carbon ketone, butanone-2.

Since the 2,4-dinitrophenylhydrazones of carbonyl compounds having the same number of carbon atoms run parallel when chromatographed with the methanol-heptane solvent, regardless of whether they are derivatives of aldehydes or ketones *(3)*, isolation and further characterization by determination of melting points of the cheese carbonyl derivatives were attempted.

The melting points of the derivatives of the cheese carbonyl compounds that were isolated by paper chromatographic fractionation and purified by recrystallization from ethanol are reported in Table 2. The lowest melting form of acetaldehyde derivative was isolated from the derivative mixture from Sample 8, whereas insufficient of fraction *a* was isolated from Sample 4 to permit purification by recrystallization. The melting points of the *b* derivative fractions indicate that acetone was present in all samples. Derivative fraction *c* present only in the distillate from Sample 1 melted at the same temperature as known

TABLE 2

Melting points of chromatographically fractionated 2,4-dinitrophenylhydrazones of the carbonyl compounds distilled from samples of blue-mold type cheese

Sample No.	Derivative fraction					
	a	b	c	d	e	f
	(° C.)	(° C.)	(° C.)	(° C.)	(° C.)	(° C.)
1	—	126.0-127.0	116.0-117.0	143.0-145.0	56.0-58.0	[a]
2	—	125.5-126.5	—	143.0-145.0	73.0-75.0	37-38 55-57
3	—	124.5-126.0	—	—	71.0-74.0	[b]
4	[a]	126.0-127.0	—	142.0-144.0	[a]	[a]
5	—	126.0-127.0	—	144.0-145.0	72.0-74.0	[a]
6	—	126.0-127.0	—	142.0-143.5	57.0-58.0 70.0-72.0	38-39
7	—	125.0-126.5	—	143.5-145.0	72.0-73.0	[a]
8	146.0-147.0	126.0-127.0	—	143.0-144.0	—	—
9	—	126.0-127.0	—	143.0-144.0	72.0-74.0	[a]
10	—	126.0-127.0	—	143.0-144.0	73.0-74.0	[b]
Known deriv.	Acetaldehyde	Acetone	Butanone-2	Pentanone-2	Heptanone-2	Nonanone-2
Observed m.p.	147.0-148.0	126.0-127.0	116.0-117.0	143.0-144.0	73.5-74.0	58.0-59.0
Reported m.p.	148 157 (4) 168.5	126 (4)	116-117 (4)	143-144 (4)	74 (8) 89 (4)	—

[a] Insufficient derivative isolated to permit purification.
[b] Not separable from adjacent *e* and *g* fractions.

butanone-2 derivative. The presence of pentanone-2 in nine of the ten cheese samples was confirmed by the melting points of the *d* derivative fractions. Although no difficulty was experienced in separating fractions *d* and *e* in one fractionation, it required two fractionations to remove all traces of fraction *f* from fraction *e*, as judged by the appearance of the chromatograms. In most instances the melting points of the *e* fractions thus isolated and recrystallized agreed fairly well with that for known heptanone-2 derivative (73.5°-74.0° C.). However, the initial melting points of the twice recrystallized *e* fractions from Samples 1 and 6 were low. In the fraction from the latter sample some melting and a partial conversion in crystal form was noted in the range of 56°-58° C. The resulting needle form dissolved in the melt at 67°-68° C. By alternately heating and cooling in the range of 60°-68° C. it was possible to enhance the conversion to the needle form. Eventually it was observed that needles isolated from the melt melted at 70°-72° C. Whether this behavior resulted from an impurity or whether there is a crystalline form of heptanone-2 derivative which melts at 56°-58° C. is not known.

In attempting to isolate the nonanone-2 derivative from the extracts from Samples 2, 5, 6, 7, and 9, it was found that even after two chromatographic fractionations it was difficult to determine on subsequent test chromatograms whether all of the adjacent heptanone-2 derivative had been removed. This was due to the tendency of the more concentrated bands of nonanone-2 derivative to tail slightly and obscure any traces of heptanone-2 derivative that might be

present. In an attempt to obviate this difficulty only the leading half of each *f* band was cut out and eluted. This, of course, reduced the yield of derivative considerably, thereby making further purification by recrystallization difficult or impossible in most cases. In the two instances where sufficient crystalline derivative was obtained from fraction *f*, the melting points observed agreed with those obtained by Patton *(6)* for the same derivative of a fraction which he confirmed was nonanone-2 by the melting point determination on the semicarbazone derivative. Despite this agreement, the present melting point data are not considered to be diagnostic in that the derivatives isolated melted at points considerably below that for the known nonanone-2 derivative. No melting point for this derivative has been found in the literature.

Sufficient fraction *g* was isolated in Samples 3 and 10 to permit further chromatographic verification of its identity. Spots of this fraction ran with those of known undecanone-2 derivative when chromatographed with this and the nonanone-2 derivative. No attempt was made to determine the melting point of this fraction, since the quantity available precluded further purification by recrystallization.

Comment. A comparison of the chromatogram in Figure 1 and the organoleptic judgments in Table 1 reveals that all samples possessing a ketonic aroma typical of Blue cheese contained pentanone-2, heptanone-2, and nonanone-2. There was considerable variation in the concentration of these ketones between cheeses, which accounted for some variation in the intensity of the typical aroma. However, there was no discernible variation in the quality of the ketonic aroma between samples that might be attributable to the apparent variation in the relative concentration of these ketones within cheese samples. The limited number of samples did not permit detection of any consistent differences in aroma or ketone content between cheese varieties. Sample 4 had the best flavor despite the fact that its content of the critical ketones was relatively low. Some rather specific flavor defects in other samples masked, or certainly detracted from, that part of the flavor which is due to the presence of the desirable methyl ketones. Of the three samples possessing an atypical aroma, Samples 1 and 3 had an earthy odor and flavor, which was probably due to contamination or invasion of the cheese with undesirable molds. This was visibly evident in Sample 1. Sample 8 yielded no heptanone-2 or nonanone-2 and had little or no aroma. This was correlated with the lack of sufficient mold growth in this cheese.

DISCUSSION

Although the paper chromatographic separation of the 2,4-dinitrophenylhydrazones of methyl ketones above heptanone-2 appears to be insufficient to permit adequate fractionation for preparative isolation, the derivative mixtures from cheese distillates are sufficiently resolved to permit identification on spot chromatograms. The fact that the 2,4-dinitrophenylhydrazones of the higher methyl ketones are not particularly good derivatives for characterization by melting point determination, especially when micro quantities are involved, probably also contributed to the difficulty experienced in conformation of the chromato-

graphic identity of these derivatives. Since these ketones have been isolated and identified by others on a macro scale from mold-ripened cheese *(6)* and other lipid substrates *(10)*, a reasonable degree of certainty may be placed on the identity of these compounds as determined by the present chromatographic procedure.

Although the conversion of all individual fatty acids from butyric to myristic to the corresponding methyl ketones by pure cultures of *P. glaucum* has been reported *(1)* and undecanone-2 has been isolated from rancid oil *(10)*, the present work appears to be the first instance in which undecanone-2 has been detected in mold-ripened cheese. Since it was detected in trace amounts in only two out of 10 samples and its presence was not correlated with any remarkable differences in ketonic flavor, it is questionable whether this ketone is of any great significance in Blue cheese flavor.

The presence of butanone-2 in Sample 1 warrants some discussion. The conversion of valeric acid to butanone-2 by *P. glaucum* has been reported *(1)*, and it is probable that *P. roqueforti* could effect the same conversion if provided this substrate. Since valeric acid is not normally present in natural fats, it is doubtful if the butanone-2 could have arisen from this source. It is probable that the valeric acid was formed during the ripening of this cheese through the metabolic activity of a contaminating organism, the presence of which was suggested by the flavor and appearance of the sample.

The presence of traces of acetaldehyde in the distillates from two samples was not unexpected. This compound has been isolated from *Streptococcus lactis* milk cultures *(5, 12)*, which suggests that acetaldehyde may not be a product of mold metabolism but is formed during the curd-making process.

The methyl ketone content is, of course, not the only criterion of Blue cheese flavor, but the method described for the detection of these compounds should have considerable utility as an adjunct to organoleptic evaluation of Blue cheese flavor.

SUMMARY

A simple procedure is described for the detection of the various methyl ketones in blue-mold type cheese which entails vacuum distillation of relatively small samples and paper chromatographic separation of the mixed 2,4-dinitrophenylhydrazone derivatives formed in the distillates.

Application of the method in the examination of 10 different samples of cheese, including French Roquefort, Danish and Domestic Blue, and Gorgonzola, revealed that acetaldehyde, acetone, butanone-2, pentanone-2, heptanone-2, nonanone-2, and undecanone-2 may be present in this type of cheese. A single sample possessing little or no desirable aroma contained no heptanone-2 or nonanone-2. These, as well as pentanone-2 and acetone, were always present in cheeses possessing a typical aroma. Only traces of undecanone-2 were detected in two samples.

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WASHED AND NONWASHED BUTTER. I. FLAVOR QUALITY AND CURD CONTENT[1]

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The washing of butter granules during the manufacture of butter is a well-established practice. The objects are to remove the free buttermilk and when necessary to chill the butter granules. Removal of buttermilk is generally considered to improve keeping quality and to remove undesirable flavors when poor quality cream is used. Cold water washing is sometimes necessary when the granules are too soft for normal working and it also assists in preventing crumbly butter when the fat has a high melting point. If the water supply is of questionable quality, however, Bouchard *(2)* in 1907 recommended that butter made from sweet cream should not be washed.

In 1920, Wyant *(14)* made comparable churnings of washed and nonwashed butter and stated that the keeping quality of washed butter was slightly better than that of nonwashed butter. He did not present any data to substantiate his statement, however. Reports from Denmark *(5)* in 1939 indicated that the keeping quality of nonwashed butter was equal to that of washed butter. Jensen *et al. (6)* confirmed the Danish results in 1942. O'Shea and Lyons *(11)* in 1945 studied the effect of the curd content on the keeping quality of butter and found that large-grain butter (0.92% curd) was usually better in flavor and aroma after a period of storage than small-grain butter (0.49% curd). In 1946, Pont *(12)* found that after 3 months storage at 16° F., washed butter was slightly superior to nonwashed butter. This was not confirmed in further studies in 1948 *(13)*. The Danish conclusions have been substantiated by other investigators in Canada *(1)*, Australia *(7, 10)*, and New Zealand *(4, 8)*.

Some of the above studies also indicated that when cream of relatively poor quality was used, the keeping qualtiy of washed and nonwashed butters was similar. None of these studies, however, has correlated cream acidity with keeping quality of washed and nonwashed butters. Furthermore, the manufacturing and storage conditions used in previous studies in other countries do not reproduce Canadian methods.

The project reported here was initiated to obtain factual data under a variety of Canadian conditions. The object was to determine the effect of the omission of washing in butter manufacture on the flavor of butter, both initially and after storage, and on the curd content. It was desired also to determine if butter quality would be influenced by the initial quality of the cream.

EXPERIMENTAL PROCEDURE

Twenty-nine creameries across Canada were selected by Provincial Dairy Commissioners to cover a wide range of cream quality and, if possible, varia-

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tions in the quality of the water supply. Churnings of washed and nonwashed butter from split vats of cream were made monthly from June to November, 1953, under the supervision of provincial dairy inspectors. The standardization of moisture for nonwashed butters was made with buttermilk from the churning. Creameries were instructed to salt the butter at 2%. Six 7-lb. samples were packed from each churning and thoroughly chilled in a sharp freezer. Five boxes were sent to Toronto, Ontario, for grading and storage, and one to Ottawa, Ontario, for initial grading and analysis. Most samples were shipped without refrigeration, and with a few exceptions during warm weather they arrived in satisfactory condition. On arrival the butter was stored at –5° F.

Cream quality. In keeping with the original plan, there was a wide variation in the acidity of the cream between plants, but within most plants the acidity of the cream was fairly uniform throughout the season. The acidity of the cream on receipt ranged from 0.11 to 0.58%. Various soda compounds were used in neutralizing. Churning acidities varied from 0.07 to 0.24% but 64.7% ranged from 0.10 to 0.16%.

Grading. Each month for 6 months, a fresh sample of each churning was graded. Samples used for initial grading and sampling were returned to cold storage for 10 months, held for 2 weeks at 50° F. to simulate conditions of retail distribution, and regraded. Gradings were done by two experienced dairy produce graders who had no prior knowledge of the type of butter. Scoring was according to Canadian grade standards *(3)*, except that the designations "plus" (+) and "liner" (L) were used to indicate slight differences in flavor.

TABLE 1

Effect of storage on the distribution of butter samples according to flavor score after 6 months at –5° F.

Initial flavor score	No. of churnings	Flavor score after storage						
		40	39+	39	39L[a]	38	37	36
		(No. of churnings)						
		Washed butters						
40	8	1	2	5	—	—	—	—
39+	17	—	3	13	1	—	—	—
39	98	—	1	72	20	5	—	—
39L	33	—	—	15	10	6	1	1
38	14	—	—	—	—	9	3	2
Total	170	1	6	105	31	20	4	3
%		0.6	3.5	61.8	18.2	11.8	2.4	1.7
		Nonwashed butters						
40	8	—	3	4	1	—	—	—
39+	15	—	1	13	1	—	—	—
39	90	—	1	67	18	4	—	—
39L	42	—	—	12	17	11	1	1
38	15	—	—	—	1	9	3	2
Total	170	—	5	96	38	24	4	3
%		—	2.9	56.5	22.4	14.1	2.4	1.7

[a] L = liner.

Analysis. The butters were analyzed for fat, moisture, salt, and curd by the modified Kohman method *(9)*. Microbiological and additional chemical determinations, which were made on these butters, will be reported in later papers.

RESULTS

Flavor score. Tables 1, 2, and 3 show the change during storage of the flavor score of the washed and nonwashed butters. The flavor score of most samples with an initial flavor score of 40 and 39 + decreased on storage, but none of these samples was graded below 39 L after storage for 6 months, and only one sample of washed butter after 10 months plus 2 weeks at 50° F. On the other hand, the greater percentage of the samples with an initial flavor score of 39 and 39 L (74%) and with a score of 38 (65.5%) maintained initial flavor score after 6 months at –5° F. This proportion, however, decreased after 10 months plus 2 weeks at 50° F. The average loss in flavor score for the washed and nonwashed butters was, respectively, 0.21 and 0.22 point at 6 months, and 0.31 and 0.34 point at 10 months plus 2 weeks. Table 4 shows that most comparable washed and nonwashed churnings had the same flavor score at all gradings. Differences in flavor score were not statistically significant at initial grading ($X^2 = 1.580$ for d.f. = 4), at 6 months grading ($X^2 = 2.568$ for d.f. = 6), and at grading after 10 months plus 2 weeks ($X^2 = 2.986$ for d.f. = 7).

The effect of cream acidity on the flavor score of the butters is presented in Table 5. Initial cream acidity had a significant effect on the flavor score of

TABLE 2

Effect of storage on the distribution of butter samples according to flavor score after 10 months plus 2 weeks at 50° F.

Initial flavor score	No. of churnings	Flavor score after storage							
		40	39+	39	39L[a]	38	37	36	35
		(No. of churnings)							
		Washed butters							
40	8	1	1	5	1	—	—	—	—
39+	17	—	2	13	1	1	—	—	—
39	98	—	2	48	34	12	2	—	—
39L	33	—	—	5	13	13	2	—	—
38	14	—	—	—	1	8	3	1	1
Total	170	1	5	71	50	34	7	1	1
%		0.6	2.9	41.8	29.4	20.0	4.1	0.6	0.6
		Nonwashed butters							
40	8	1	2	4	1	—	—	—	—
39+	15	—	2	10	3	—	—	—	—
39	90	—	—	42	39	8	1	—	—
39L	42	—	—	4	16	20	2	—	—
38	15	—	—	—	1	8	4	1	1
Total	170	1	4	60	60	36	7	1	1
%		0.6	2.3	35.3	35.3	21.2	4.1	0.6	0.6

[a] L = liner.

TABLE 3

Percentage of samples losing flavor score on storage

Initial flavor score	After 6 months at –5° F.			After 10 months at –5° F. and 2 weeks at 50° F.		
	Washed	Nonwashed	Total	Washed	Nonwashed	Total
40	87.5	100.0	93.8	87.5	87.5	87.5
39+	82.4	93.3	87.5	88.2	86.7	87.5
39	25.5	24.4	25.0	49.0	53.3	51.1
39L	24.2	31.0	28.0	45.5	52.4	49.3
38	35.7	33.3	34.5	35.7	40.0	37.9
Total	35.3	36.5	35.6	52.9	56.5	54.7

butters after storage for 6 months ($X^2 = 22.595$ for d.f. = 8), but not after storage for 10 months plus 2 weeks ($X^2 = 6.173$ for d.f. = 8).

Curd content. The average composition of the butters was:

	Moisture	Fat	Salt	Curd
Washed	15.61%	81.59%	2.02%	0.78%
Nonwashed	15.49%	81.20%	2.01%	1.31%

The effect of nonwashing on the curd content of butter is shown in Table 6. The average curd content of the nonwashed butters was greater than that of the washed butters by 0.53%, with ranges of 0.15 to 0.85% for individual comparable churnings, and 0.35 to 0.77% for individual creameries.

DISCUSSION

This study has established that under Canadian commercial conditions, with a wide variety of cream qualities (0.11 to 0.58% acidity), the flavor quality of nonwashed butter is as good as that of washed butter. During storage for 6 months at –5° F. and for 10 months at –5° F. plus 2 weeks at 50° F. the average flavor score of the butters decreased, but similarly for both types of butter. These results agree with those of investigators from Denmark, Australia, and New Zealand, and with those of Arnott *et al. (1)* from Canada. That there was

TABLE 4

Summary of comparable churnings with the same and different flavor scores

Flavor score	At initial grading		After 6 months at –5° F.		After 10 months at –5° F. + 2 weeks at 50° F.	
	No. churnings	%	No. churnings	%	No. churnings	%
Same flavor score	140(157)[a]	81.8(92.4)	152(163)	89.4(95.9)	147(159)	86.5(93.5)
Washed higher than nonwashed	20(8)	12.3(4.7)	18(7)	10.6(4.1)	18(7)	10.6(4.1)
Nonwashed higher than washed	10(5)	5.9(2.9)	0(0)	0.0(0.0)	5(4)	2.9(2.4)

[a] The figures in parentheses are the results when 39 and 39 liner flavor score butters are considered to be of similar quality.

TABLE 5

Effect of cream acidity on the flavor score of washed and nonwashed butters

Cream acidity	Per cent samples losing flavor score					
	After 6 months at −5° F.			After 10 months at −5° F. and 2 weeks at 50° F.		
	Washed	Nonwashed	Total	Washed	Nonwashed	Total
(%)						
0.2 and less	50.0	50.0	50.0	50.0	54.5	52.3
0.2-0.3	26.9	26.9	26.9	61.5	53.8	57.7
0.3-0.4	28.3	28.3	28.3	50.0	53.3	51.7
0.4-0.5	32.6	40.4	37.2	46.8	57.4	52.1
More than 0.5	60.0	53.3	56.7	73.3	73.3	73.3

no significant difference in the keeping quality of the washed and nonwashed butter may be attributed in part to modern methods of production, neutralization and pasteurization, the use of stainless steel equipment, and modern refrigerated storages.

The body and texture of the butters were generally free of major criticisms. Traces of free moisture and slight unevenness in color were noticed in some butters but were no more apparent in the nonwashed than in the washed butters. Slight color defects in the form of white specks or streaks and traces of deep yellow spots were noticed in a few nonwashed butters. As buttermakers gained experience in making nonwashed butter, these slight defects were corrected.

The difference in curd content between the nonwashed and the paired washed butters showed considerable variation in this study. The unusually high curd content of some washed butters is attributed to limited washing because of a scanty or a poor quality water supply. The abnormally low curd content of some nonwashed butters is attributed mainly to a relatively high dilution of the cream from can rinsings. The curd content of the butters may also have been influenced by the size of the granules and the thoroughness of draining the buttermilk, as shown by O'Shea and Lyons *(11)*. The gain of 0.53% in the curd content of butter due to nonwashing is in the range of the gains reported by other workers. Jensen *et al. (6)* found 0.60%; Pont *(13)*, 0.44%; Lebsanft *(7)*, 0.25%; and McDowall *et al. (8)*, 0.38% for commercial butters, whereas Arnott *et al. (1)* found 0.7% and the Danish workers 0.9% for experimental butters.

TABLE 6

Curd content of washed and nonwashed butters

	Washed		Nonwashed		Difference of av.
	Average	Range	Average	Range	
	(%)	*(%)*	*(%)*	*(%)*	*(%)*
All butters	0.78	0.42-1.24	1.31	0.93-1.69	0.53
Individual creameries (av. of 6 churnings)					
Minimum	0.51	0.45-0.61	1.09	0.93-1.29	0.35
Maximum	1.12	0.96-1.24	1.48	1.39-1.56	0.77

In theory, if we assume a cream serum of 9% nonfat solids and a butter with 15.9% moisture derived entirely from the cream serum, the calculated curd content would be $\frac{15.9 \times 9.0}{91.0} = 1.57\%$.

In addition to the gain in yield, there are other important economic advantages in eliminating the washing of butter. Water is conserved, waste disposal is reduced, refrigeration costs are lower, and time and labor are reduced generally.

Under practical conditions where the cold wash water has been relied on to "firm" the granules for proper working, it may be necessary to lower the churning temperature to obtain the desired firmness in the butter granules for the proper working of nonwashed butters. Practical experience, however, has indicated that churning temperatures lower than normally used are not always necessary to obtain good physical characteristics in the butter.

SUMMARY

A study was made of the flavor quality, before and after storage, and of the curd content of 170 comparable churnings of washed and nonwashed butters. A wide variety of cream qualities was covered. The butters were manufactured under commercial conditions in 29 Canadian creameries.

There was no significant difference in the flavor quality of the comparable washed and nonwashed butters before and after storage.

The flavor quality of the butter was highly dependent on the initial cream acidity after 6 months at –5° F., but not after 10 months at –5° F. plus 2 weeks at 50° F.

The yield of butter was increased as a result of nonwashing because of the higher curd content (av. 0.53%).

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A METHOD FOR DETERMINING TOTAL DIGESTIBLE NUTRIENTS IN GRAZED FORAGE

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The method developed by Reid and coworkers *(4)* to estimate the digestibility of the dry matter consumed by grazing animals has been important in stimulating much needed pasture research. The most important contribution of this technique is that, when used with total fecal collection, it permits one to estimate the dry matter intake of a grazing animal. It is probable, however, that under most conditions the total digestible nutrient (TDN) content would be a more reliable measure of the productive value of the dry matter consumed than would dry matter digestibility.

In the conventional method of determining TDN it is necessary to know the composition of the dry matter consumed with respect to crude protein, crude fiber, ether extract, and nitrogen-free extract (NFE). It has been shown, however, that TDN can be accurately determined from the digestibility of the organic matter of the feed *(2)*. It is therefore unnecessary to determine the individual components of the organic matter in this method. It is necessary to know only the ether extract and organic matter content of the feed and the organic matter of the feces. One of the difficulties in pasture research has been the problem of measuring the intake of the proximate constituents of the consumed forage. Because of selective grazing a proximate analysis of a clipped forage sample may bear little relationship to that being consumed *(1)*. It is possible, however, that when all the organic constituents are lumped together and determined merely as total organic matter, the analysis of a clipped sample may closely approximate that of the grazed forage and be of value in estimating the digestibility of the organic matter. Hardison *et al. (1)* presented data which showed that, on the average, grazed samples contained approximately 23.1% more protein, 38.7% more ether extract, 16.6% less crude fiber and 2.5% less NFE than clipped samples. The total organic matter, however, differed only 1.9%. This should be well within the accuracy of the method for estimating the dry matter intake. It appears probable, therefore, that the organic matter of a clipped sample of forage may be used to estimate organic matter digestibility from the formula:

$$(1) \qquad D = 100 - \left(\frac{100AY}{BX} \right)$$

where D is the digestion coefficient for organic matter, A is the organic matter per gram of dry matter in the feces, B is the organic matter per gram of dry matter in the forage, X is the chromogen per gram of dry matter in the feces, and Y is the chromogen per gram of dry matter in the forage. Y is estimated

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from X by the formula of Reid *et al. (4)*. When the digestion coefficient for organic matter is known, it is then possible to determine TDN by applying a factor calculated by the formula:

$$(2) \qquad F = M\ (0.01 + 0.000125\ E)$$

where M is the per cent organic matter in the dry matter of the feed and E is the ether extract as per cent of the organic matter *(2)*. It can readily be seen that one must obtain E as well as M from a clipped sample. M is merely B times 100 in formula 1, and the accuracy of this term has been discussed. The question then arises as to the influence of a variation in the ether extract on the accuracy of the conversion factor, F. In the data of Hardison *et al. (1)*, cited previously, the organic matter *(M)* and ether extract as per cent of the organic matter *(E)* for the average of all grazed samples are 91.1 and 4.7, respectively. For the clipped forage the two values are 92.9 and 3.3. F, calculated from these two sets of values, therefore, becomes 0.965 for the grazed forage and 0.967 for the clipped forage. If these factors are applied to an assumed organic matter digestion coefficient of 60%, the TDN would be 57.9% and 58.0%, respectively. It does appear possible, therefore, to use the organic matter and ether extract content of clipped forage in a method for determining the TDN of grazed forage by combining the method of Reid *et al. (4)* and that of Lofgreen *(2)*.

EXPERIMENTAL PROCEDURE

A pasture utilization study was set up at the University farm at Davis during the months of May through September of 1954. The details of the layout of the field and the various management practices have been described in an earlier publication *(3)* and will not be repeated completely herein. Only the procedures having direct bearing on the problem being considered will be discussed.

Nine steers weighing approximately 550 lb. each were placed on a pure stand of alfalfa and grazed according to a 5-day rotational system. Nine similar steers were used to study a 1-day rotational system, sometimes called strip grazing. A third lot, consisting of ten steers, was placed in the dry lot and fed alfalfa soilage harvested twice daily with a field chopper from a section of the same field used by the two other groups. Three steers in each lot were fitted with collection harnesses and bags, and collection of total feces was made every other 5-day period. Nine 5-day collections were made. Collections were made once daily and were weighed and sampled for dry matter, ash, and chromogen analyses.

During one of the 5-day periods samples were hand plucked from the rotational pasture each day. An effort was made to pluck the most immature material available to the animal. Chromogen analyses were made by the method of Reid *et al. (4)* after exposing the extract to 800 foot-candles of light for 20 minutes. Such a treatment resulted in a stable solution, which changed little in transmission if stored in the dark. The transmission of a standard chromogen solution was determined on Reid's spectrophotometer, and the standard curve was adjusted so that Reid's formula could be used for predicting the concentration of chromogens in the feed from that in the feces.

RESULTS AND DISCUSSION

A comparison of the analysis of the daily plucked samples with that of the soilage during the same period is presented in Table 1. The purpose of these samples was to determine if the analysis for organic matter and ether extract (*M* and *E* in formula 2) would result in a difference in the conversion factor, *F*, when compared to that calculated from the analysis of the alfalfa soilage fed to the dry lot animals during the same period. If a large difference occurred in either *M* or *F*, the TDN estimated by the described method would be of questionable value. On the other hand, if no marked differences occurred, one could be reasonably sure that the method would give a good estimate of the TDN content of grazed forage. It is interesting to note that the *F* values calculated

TABLE 1

Comparison of plucked forage with clipped forage in the same period

Day	Dry matter at time of sampling	Organic matter on dry basis (M)	Ether extract as per cent of organic matter (E)	Conversion factor (F)
	(%)	*(%)*	*(%)*	
		Plucked samples		
1	19.6	91.2	2.3	
2	20.8	90.9	2.8	
3	21.1	90.0	2.8	
4	24.0	90.8	2.5	
5	26.6	90.3	2.4	
Means	22.4	90.6	2.6	0.935
		Soilage sample		
Composite	21.9	91.2	2.2	0.937

from the two sets of values are almost equal even though the organic matter of the soilage (clipped) sample is higher and the ether extract lower than those of the plucked samples. This is the same situation which occurred in a comparison of the grazed and clipped samples of Hardison *et al. (1)* discussed earlier. It is reasonably certain, therefore that the *F* value calculated from the clipped forage is satisfactory for the purpose of determining TDN in the grazed forage.

As a further indication of the validity of the method the digestibility of the organic matter *(D)* and the TDN as determined from the fecal composition of the three steers on the 5-day rotational pasture as presented in Table 2 should be noted. The digestibility of the organic matter *(D)* and the TDN differ only slightly with the two sets of values.

Table 3 presents a comparison of the dry matter digestibility and the TDN content of the pastured alfalfa with that fed by the soiling method. Since there was no significant difference between the rotation and strip grazing methods, these two treatments are combined under the heading of "pasture." It will be noted that there was no difference in the digestibility of the dry matter when

TABLE 2

Comparison of the digestibility of the organic matter and TDN by use of hand-plucked samples and soilage (clipped) samples

Steer No.	See formula (1)					See formula (2)	TDN as per cent of dry matter
	A	B	X	Y	D	F	
				Hand-plucked samples			
15	0.872	0.906	337	136	61.1	0.935	57.1
24	0.861	0.906	341	136	62.1	0.935	58.1
30	0.854	0.906	351	140	62.4	0.935	58.3
Means					61.9		57.8
				Soilage sample			
15	0.872	0.912	337	136	61.4	0.937	57.5
24	0.861	0.912	341	136	62.3	0.937	58.4
30	0.854	0.912	351	140	62.6	0.937	58.7
Means					62.1		58.2

fed as pasture or soilage. On the other hand, there was a small but highly significant difference in the TDN of the forage consumed by the two methods. Since it is logical to assume that the animals on pasture had more opportunity to select their forage than those fed soilage, one would expect a higher TDN in the forage consumed by the animals on pasture.

The results of the dry matter digestibility do not agree with those of Hardison *et al. (1)*, who reported a higher dry matter digestibility for grazed than for clipped forage. Two factors probably can account for this apparent difference in the two trials. The stage of maturity at which the forage is grazed undoubtedly has an effect upon the degree of selection. For example, on a pure stand of brome grass at the bloom stage Hardison *et al. (1)* reported dry matter digestibility of 67% and 62% for grazed and clipped forage, whereas the digestibility of the aftermath at a vegetative stage was 60% and 61% for grazed and clipped forage, respectively. The same trend was true of a stand consisting of 80% alfalfa. Since the alfalfa grazed in the studies reported here was never more mature than early bloom, one would not expect as extensive

TABLE 3

Dry matter digestibility and TDN of alfalfa pasture and soilage

Collection period No.	Dry matter digestibility		TDN, dry basis	
	Pasture	Soilage	Pasture	Soilage
	(%)	*(%)*	*(%)*	*(%)*
1	60.1	59.1	57.8	55.7
2	60.2	58.9	58.5	56.3
3	60.1	58.6	58.8	58.4
4	58.1	57.2	55.6	53.5
5	58.8	59.3	56.5	55.6
6	58.7	58.6	56.8	55.0
7	59.9	59.5	58.4	56.9
8	59.3	59.9	58.0	56.4
9	59.0	60.2	57.6	56.0
Means	59.4	59.0	57.6**	56.1

** Significant at 1% level.

selective grazing. It is interesting to note that the dry matter digestibility of the grazed forage for periods 1 through 4 was higher than that of the clipped forage, although there was no difference thereafter. The records show that during the early periods the alfalfa was in early bloom but in the later ones it was in the bud stage. An additional factor in these trials is that the soilage animals had free access to forage twice daily and, although the material was chopped, some selection could be practiced. This was shown by later digestion trials with sheep, in which it was found the soilage weighback had considerably less TDN than that offered the animals. This would tend to bring the digestibility of the clipped forage closer to that which had been selected by the grazing animals.

The probable reason for the failure of the dry matter digestibility to show evidence of selective grazing whereas the TDN did is that the ash content does not play a part in TDN determination but it does in the determination of the dry matter digestibility. The dry matter in the feces of the pasture steers contained an average of 3.5% less organic matter than the feces of those fed soilage. If it is assumed that the dry matter of a grazed forage sample contains an average of 1.9% less organic matter than a clipped sample *(1)*, it can readily be seen that the dry matter of the feces from grazing steers would contain less organic matter in relation to the grazed forage than would the feces of steers fed clipped forage in relation to the organic matter of the clipped sample. This would result in a higher digestibility of the organic matter for grazed forage even though there may be no difference in the dry matter digestibility. This would then be reflected in a higher TDN content of the grazed forage.

It appears from the data presented that the method discussed for the determination of the TDN content of grazed forage shows promise of providing useful information in pasture studies.

SUMMARY

A method is presented for the determination of the TDN content of grazed forage. The digestibility of the organic matter is determined by the chromogen ratio technique by use of the formula:

$$D = 100 - \left(\frac{100AY}{BX} \right)$$

where D is the digestion coefficient for organic matter, A and B are the organic matter per gram of dry matter in the feces and forage, respectively, and X and Y are the chromogen per gram of dry matter in the feces and forage, respectively. TDN is determined by multiplying the digestion coefficient for organic matter, D, by a conversion factor calculated by the formula:

$$F = M\ (0.01 + 0.000125\ E)$$

where M is the per cent organic matter in the dry matter of the feed and E is the ether extract as per cent of the organic matter. Data are presented in support of the validity of the method.

Evidence is shown which indicates that selective grazing may be more pronounced with mature than with immature forage.

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THE SUPPLEMENTAL VALUE OF ALFALFA HAY WHEN FED TO COWS ON PASTURE[1]

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The mid- or late-summer slump in milk production is a common seasonal occurrence in most states. Efforts to prevent or minimize the reduction in milk production have followed different routes, including the feeding of hay as a supplement to pasture.

After observing *(10)* that cows in Louisiana, during warm weather, spent a major portion of each day in the shade, two trials *(11)* were conducted by Seath and Miller in which hay was fed as a supplement to pasture in an effort to prevent the mid-summer decline in milk production. In the preliminary trial, conducted in 1940, insignificant increases in milk production were secured when milk cows were fed 9 lb. of white Dutch clover hay daily. In the second trial two levels of hay feeding were tried, i.e., 0.5% of body weight daily and ad libitum feeding. Hay consumption averaged 4.69 lb. daily for the first plan and 5.54 lb. daily for the second plan. Milk production was significantly increased when hay was full fed. Under both plans hay was found to be economically unprofitable.

A Kentucky trial *(1)* showed no appreciable increase in milk production or body weights when alfalfa hay was fed free choice to milking Holstein and Jersey cows on pasture during July and August of 1948.

In 1950 Seath and Elliott *(9)* found in a short trial that feeding either corn silage or an additional amount of grain mixture to dairy cows during the summer months aided in preventing the late summer decline in milk production. However, the benefits were not large enough to be economically profitable, although this may have been due to better than average grazing conditions.

In 1952 Seath reported data taken from a short trial *(8)* showing the effects of supplementing pasture with either alfalfa silage or additional grain. Some improvement in the level of milk production was noted when silage was fed to cows grazing a mixture of Kentucky 31 fescue and ladino clover and when extra grain was fed to cows grazing straight Kentucky 31 fescue.

A report by McCullough *et al.* in 1953 *(5)* showed that high quality legume hay may have helped to maintain a high level of summer milk production when fed as a supplement to cows on a Kentucky 31 fescue pasture.

In view of the meager data available on this subject, an experiment was conducted in 1954 in an effort to measure what influence alfalfa hay as a supplement, when fed to cows on summer pasture, would have on their milk production, body weight, dry matter intake and the digestibility of the ration.

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[1] The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director.

EXPERIMENTAL PROCEDURE

Sixteen cows (eight Holsteins and eight Jerseys) representing eight pairs of milking cows were used in this experiment. Before the cows were grouped into pairs, consideration was given to breed, age, stage of lactation, and milk production. One cow from each pair was assigned at random to receive the hay and the other to serve as a control.

During the period May 20 to September 8 the cows were allowed to graze a permanent pasture consisting primarily of Kentucky bluegrass with only a scattering of white clover. The condition of the pasture varied from good to fair, as described by Morrison *(6)*, with the latter condition prevailing during the mid-summer when the herbage was relatively short and brown.

Both groups of cows received a grain ration containing 14% protein equivalent (20% of the protein was supplied by nonprotein nitrogen compounds), which was fed according to milk production with amounts based on "good" pasture as shown in Table VIIIb of *Feeds and Feeding (6)*. Adjustments in the amounts of the grain mixture fed were made monthly, based on the average percentage decline in production for both groups of cows.

The eight cows receiving hay were allowed all of the alfalfa hay (equivalent in quality to U. S. No. 2) they would consume during 2-hour periods (A.M. and P.M.) while in stanchions at milking time. Accurate weights on all hay and grain consumed and refused were kept for each cow.

Digestibility trials and measures of dry matter intake were conducted on all cows every 2 weeks by combining the fecal chromogen(s) *(7)* and the chromic oxide *(4)* techniques. Each cow received daily 15 g. of chromic oxide in a gelatin capsule. Fecal "grab" samples were collected at 6:00 A.M. and 4:00 P.M. daily for 5 days (Saturday through Wednesday) of each 2-week test period. These samples were composited and analyzed for dry matter, chromogen, and chromic oxide. The feces were analyzed for chromium oxide by the method outlined by Gehrke and Becker *(3)*, which is a modification of the method published by Bolin *(2)*. Grass samples for dry matter content were gathered on Monday of each test period.

EXPERIMENTAL RESULTS

Dry matter intake and digestibility. The intake of dry matter by the cows as furnished by grain, hay, and pasture and the dry matter digestibility of the entire ration are shown in Table 1. Examination of the data shows that appreciable amounts of dry matter were consumed from the alfalfa hay by the hay-supplemented group, varying from 4.7 to 17.1 lb. and averaging 8.2 lb. per cow daily. The amount of dry matter furnished by the grain mixture was almost identical for the two groups, averaging 10.0 lb. for those receiving hay and 9.8 lb. for those not fed hay. The intake of dry matter from hay apparently caused a highly significant decrease ($P < 0.01$) in the dry matter intake from pasture, with those receiving hay averaging 11.4 lb. and those without hay, 16.8 lb. per cow daily. The hay-fed group, however, averaged 29.6 lb. for total dry matter

TABLE 1

Dry matter intake and digestibility for cows with and without hay as supplement as determined by the chromogen(s) and chromic oxide techniques

Period	Dry matter								
	Hay as supplement					No hay as supplement (control)			
	Intake from					Intake from			
	Grain	Hay	Past.	Total	Dig.	Grain	Past.	Total	Dig.
	(lb.)	*(lb.)*	*(lb.)*	*(lb.)*	*(%)*	*(lb.)*	*(lb.)*	*(lb.)*	*(%)*
I 6/12 – 6/16	10.9	4.9	11.5	27.3	56.3	10.2	13.8	24.0	58.2
II 6/26 – 6/30	11.7	5.5	11.2	28.4	56.8	10.7	14.5	25.2	56.5
III 7/10 – 7/14	11.2	8.2	10.3	29.7	54.0	11.1	13.2	24.3	54.1
IV 7/24 – 7/28	8.7	17.1	6.8	32.6	53.7	8.8	19.5	28.3	53.6
V 8/7 – 8/11	8.6	9.0	9.0	26.6	56.4	8.3	19.0	27.3	61.5
VI 8/21 – 8/25	8.9	4.7	19.4	32.8	65.1	9.5	21.0	30.5	67.5
Av.	10.0	8.2	11.4	29.6	57.0	9.8	16.8	26.6	58.6
Change over no hay *(%)*	—		−32.1	+11.2					

consumed daily per cow as compared to 26.6 lb. for the nonhay-fed group. Thus, in spite of a decrease of 5.4 lb., or 32.1%, in amount of dry matter consumed from pasture by this group as compared to the no-hay group, the total dry matter intake exceeded that for the other group by 3.0 lb. per cow daily, or 11.2%. This difference was statistically significant ($P < 0.05$).

Determinations as to apparent digestibility of the entire ration (columns 5 and 9) show no consistent difference among rations, although for some of the

TABLE 2

The influence of feeding hay to cows on pasture on yield of FCM with rainfall by weeks

Expt. week	Dates (1954)	No. of cows	Av. daily production of FCM			Rainfall
			Control group	Hay group		
		(pairs)	*(lb.)*	*(lb.)*	*(% of control)*	*(in.)*
−1	5/20 – 5/26	8	42.9	43.6	101.6	0.24
		(6)	(40.9)	(42.2)	(103.2)	
1	5/27 – 6/2	8	41.5	40.8	98.3	0.79
2	6/3 – 6/9	8	44.4	43.5	98.0	0.93
3	6/10 – 6/16	8	45.0	39.7	88.2	0.95
4	6/17 – 6/23	8	39.2	39.8	101.5	0.61
5	6/24 – 6/30	8	38.2	36.4	95.3	0.10
6	7/1 – 7/7	8	35.2	34.9	99.1	0.17
7	7/8 – 7/14	8	34.5	33.0	95.6	1.22
8	7/15 – 7/21	8	32.7	33.8	103.4	2.76
9	7/22 – 7/28	8	32.1	35.6	110.9	—
10	7/29 – 8/4	8	33.7	34.2	101.5	2.19
11	8/5 – 8/11	8	35.0	36.8	105.1	1.40
12	8/12 – 8/18	(6) [a]	(34.0)	(38.8)	(114.1)	0.07
13	8/19 – 8/25	(6)	(34.6)	(34.3)	(99.1)	0.13
14	8/26 – 9/1	(6)	(31.5)	(34.0)	(107.9)	0.17
15	9/2 – 9/8	(6)	(31.6)	(31.7)	(100.3)	0.36
11-week av.		8	37.5	37.2	99.2	
15-week av.		(6)	(37.3)	(38.8)		

[a] Three cows were dropped from the experiment because of advanced lactation, thus making it necessary to omit two pairs in comparison for last 4 weeks.

experimental periods and for averages of all periods a slightly higher digestibility was found for the nonhay ration than for the hay-supplemented ration. No significance is attached to the small differences found, however.

Milk production. Yields of fat-corrected milk (FCM) for weekly periods, as shown in Table 2, give evidence of no uniform advantage or disadvantage in favor of the group of cows fed hay. The eight hay-fed cows produced during the week prior to the experimental period an average of 0.7 lb. per cow daily, or 1.6% more than the eight control cows. During the 11th experimental week they averaged 1.8 lb. per cow daily, or 5.1% higher in production than the control cows. There were, however, six of the 11 weeks when the production of the control cows had a higher average than that of the hay-fed cows, and the actual 11-week average was highest for the control group. No statistically significant differences were found between the production of the two groups (eight pairs) during the 11-week period. Six cows in each group (six pairs) continued on the experiment for 15 weeks. Two pairs were discontinued owing to advanced lactation periods. The production for the six pairs (as shown in parentheses, Table 2) for the 12th-15th weeks, inclusive, gives added evidence of the inconsistent influence that hay feeding had on milk production. During the 12th and 14th weeks it appears that hay feeding may have increased production, whereas the reverse was true for the 13th and 15th weeks.

Both experimental groups averaged high in monthly (4-week) persistency of milk production with no consistent trend favoring hay feeding. During the 11-week period for all eight pairs of cows, and during the 15-week period for six pairs, the production had declined an amount to represent a 4-week persistency average of approximately 93%. This would indicate that the nutrient intake for each group of cows was sufficient to maintain a relatively high level of milk production. These results were somewhat surprising in view of the observation that the pasture was grazed rather closely and appeared to be somewhat dry and brown, especially during mid-summer.

The rainfall in Lexington by weekly periods (right-hand column in Table 2) indicates much variation in precipitation. The average weekly rainfall was 0.82 in., compared to a 60-year average of 0.97 in. Even though the rainfall was below normal, there was no apparent close relationship between the rainfall for any given week and the performance of the two experimental groups during that week or the following week. There was some indication that during midsummer, July 15 to August 18, the results of hay feeding were beneficial. Even so, it appears that the increases in milk production would need to be greater than those secured in this experiment if hay feeding were to be economically profitable.

Body weight changes. Changes in body weight (Table 2) give evidence that the 11% increase found in intake of dry matter by the hay-fed group had a highly significant influence ($P < 0.01$) on their performance. Although there were inconsistent variations from one 2-week weigh period to the next, the change for the entire period showed gains in body weight of the hay-fed group averaging 50 lb. per cow. The control group finished the experiment averaging the same

TABLE 3

Effect on body weight of feeding hay to cows on pasture

Cow No.	Initial wt. 5/24-26	Weight gain by periods *(lb.)*							Total gain
		6/15-16	6/29-30	7/13-14	7/27-28	8/10-11	8/24-25	9/8-9	
Control group									
998	859	–6	12	–8	3	–5	–51	47	–8
992	789	6	3	9	23	–8	–9	32	56
876	870	–9	6	–4	15	10	–37	30	11
835	1,029	26	–15	–62	37	–37	–27	[a]	–78
842	1,267	15	5	13	33	–12	–60	26	20
834	1,222	–22	–6	–9	17	–23	[a]	[a]	–43
990	1,204	–6	–8	8	38	–7	–51	50	24
999	756	9	6	14	–17	13	–24	17	18
Total		13	3	–39	149	–69	–259	202	0
Av.	1,000								0
Hay group									
858	791	23	–14	2	18	24	–38	28	43
968	874	–50	–2	27	5	12	4	27	23
818	940	18	12	–12	12	2	–14	32	50
936	961	25	1	24	31	14	–12	[a]	83
905	1,170	28	–8	14	41	–24	–45	54	60
824	1,396	36	–10	13	–10	27	–46	[a]	10
918	1,091	–30	45	–12	33	15	–37	56	70
899	833	13	–1	10	7	6	–3	33	65
Total		63	23	66	137	76	–191	230	404
Av.	1,007								50

[a] Animals discontinued on experiment.

as at the start. Just why the hay feeding did influence gain in body weight yet had no consistent influence on milk yield is not explainable from the evidence at hand.

SUMMARY

During the summer of 1954, eight cows while on bluegrass–white clover pasture were fed all the alfalfa hay they would eat for 2 hours twice daily, and their production was compared to that of eight comparable cows on the same pasture without hay as a supplement. Both groups were fed a 14% protein grain mixture at a moderate rate in line with milk production.

Estimates of dry matter intake by combining the chromogen(s) and chromic oxide techniques indicated that the cows receiving hay increased their intake by 11%, with the hay group averaging 29.6 lb. per cow daily and those without hay, 26.6 lb. The portion of the dry matter furnished by pasture was reduced by 32% with the hay-fed group averaging 11.4 lb. per cow daily from pasture and the no-hay groups, 16.8 lb.

Hay feeding failed to produce a significant increase in milk production, although there was a tendency for the hay-fed group to produce more fat-corrected milk than did the group without hay during the period from July 15 to August 18. During a majority of the remaining 11 experimental weeks the control group excelled the hay-fed group in average milk production.

Cows fed hay gained an average of 50 lb. per cow during the experiment, whereas the control cows' average weight was the same at the end as at the start of the experiment. The experiment produced no evidence as to why alfalfa hay as a supplement to pasture had a greater effect on body weight changes than on milk production, other than the observation that the persistency of milk production for both groups of cows was high, averaging about 93% on a 4-week basis.

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THE UTILIZATION OF SMOOTH BROMEGRASS *(BROMUS INERMIS)* UNDER ROTATIONAL AND STRIP GRAZING SYSTEMS OF PASTURE MANAGEMENT. I. ANIMAL AND PASTURE PRODUCTION[1, 2]

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Pasture management, of primary importance in its effect on the plant-animal complex, is imposed on an area whenever man utilizes the available herbage through the use of grazing livestock. Good management is designed to give the maximum efficiency of utilization commensurate with continued productivity of the pasture and the grazing animal.

The development of intensive pasture management systems has been comparatively recent. The Hohenheim system of pasture management combines intensive cultural practices with rotational grazing *(12)*. Holmes and Waite *(7)* have proposed strip grazing (daily rotational grazing or close-folding) with temporary electric fences. This system has been widely adopted in England and New Zealand and to a lesser extent in the United States.

European workers *(3, 6, 8, 9, 13, 14)* have reported 20 to 40% increases in yield from strip grazing as compared with rotational grazing. Brundage and Petersen *(1)*, using identical twins, found small differences in animal production under the two systems. A greater yield in total nutrients was returned from the strip grazing system, however, because a large portion of the total herbage was harvested as hay, whereas any excess herbage produced on the continuously grazed pasture was wasted.

The importance of proper pasture management is magnified in Alaska by the brevity of the grazing season, the extended winter feeding period, and the limited cleared land. These conditions emphasize the importance of obtaining maximum utilization of the available land, for increases in dairy farming often may be obtained more easily through an increase in the number of animal units maintained per acre than by an increase in farm size.

EXPERIMENTAL PROCEDURE

Grazing trials were conducted during the seasons of 1953 and 1954 on smooth bromegrass pasture to determine if strip grazing would result in higher efficiency of herbage utilization than rotational grazing. During 1953, 4 acres of bromegrass were used which had been seeded 5 years previously and were uniform

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[2] Data presented are from a thesis submitted by A. L. Brundage to the Graduate Faculty of the University of Minnesota in partial fulfillment of the requirements for a Doctor of Philosophy degree.

FIG. 1. Temporary electric fence reel assembly.

in appearance. During 1954, 6.4 acres which had been seeded the previous year were used. The bromegrass stand was irregular and partially infested with wild barley *(Hordeum jubatum)*.

Pasture management was the same during both trials. A permanent electric fence divided each field into two equal halves, one of which was further subdivided into four equal paddocks, which were subjected to a rotational grazing system. The remaining half of each field was grazed under a strip grazing system. Each morning, after milking, the cows were placed on a new strip of pasture, which was estimated to adequately supply their grazing requirements for 24 hours. Three temporary electric fences, each utilizing a reel assembly (Figure 1), were used. The unit was anchored in place by forcing the three legs into the ground. The wire was then unwound from the insulated reel and connected to the fence across the field. Pigtail posts insulated with rubber tubing *(1)* were then placed every 20 to 40 ft. to support the wire. One fence was used to keep the cows from progressing to ungrazed material. The second served as

TABLE 1

Dates on experimental pasture

Rotational grazing	Strip grazing
1953 TRIAL	
From 6-2 A.M. to 6-28 A.M.	From 6-2 A.M. to 7-1 A.M.
From 7-4 A.M. to 7-11 A.M.	From 7-9 A.M. to 7-14 A.M.
From 8-2 A.M. to 8-16 A.M.	From 8-1 A.M. to 8-16 A.M.
From 9-6 A.M. to 9-14 A.M.	From 9-6 A.M. to 9-14 A.M.
1954 TRIAL	
Period 1	
From 5-29 A.M. to 7-6 A.M.	From 5-29 A.M. to 7-6 A.M.
From 7-20 A.M. to 8-9 A.M.	From 7-20 A.M. to 8-9 A.M.
Period 2	
From 8-31 A.M. to 9-18 A.M.	From 8-31 A.M. to 9-18 A.M.

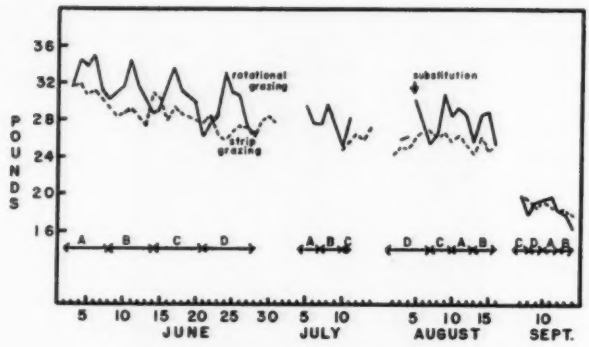

FIG. 2. Average daily FCM production, 1953 trial. Solid line—rotational, broken line—strip grazing. Arrows indicate periods when the rotational group was on specific paddocks. Breaks indicate periods when the groups were removed from the pasture.

a back fence to keep them from the previously grazed areas. The third was unwound or rewound as the strips were advanced up or down the field and formed a lane which facilitated placing the cows on the strip being grazed.

Table 1 and Figures 2 and 3 show where the groups were located at different times during the course of the trials. During the initial grazing of the first trial, the group under the rotational system of management was not moved until the other group had grazed an area equivalent in size to the rotational paddock. Subsequent movements were somewhat independent of each other. In the second trial, movement of the rotational group was always determined by the group on strip grazing. The two systems were restricted in this manner to reduce personal error and bias in rotating the pastures and allow each group an equal opportunity to utilize the available herbage on their respective half of the pasture.

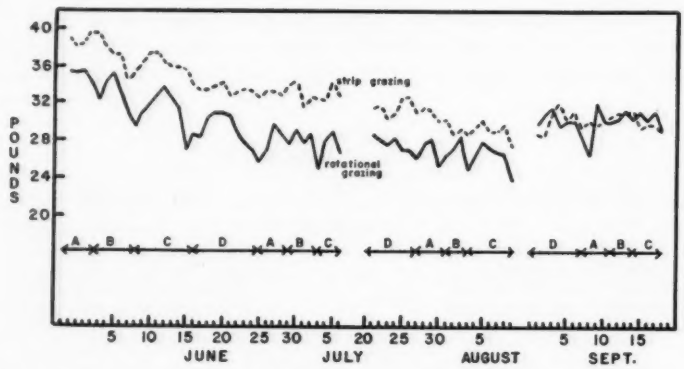

FIG. 3. Average daily FCM production, 1954 trial. Solid line—rotational, broken line—strip grazing. Arrows indicate periods when the rotational group was on specific paddocks. Breaks indicate periods when the groups were removed from the pasture.

It was necessary to remove both groups from the experimental pastures at times during both seasons to allow recovery for regrazing. At these times the animals were placed on other pastures with the remainder of the Experiment Station herd.

Four milking dairy cows were used for each management system during both trials. These groups were balanced insofar as possible according to breed, age, stage of lactation, weight, previous history, and level of production prior to the start of the experiments. Animals did not change groups during the trials. During the 1953 season, one cow on the rotational system developed acute mastitis and was replaced by another animal.

It was necessary to use four groups of cows during 1954. The two groups initially used were unavailable for grazing the pastures in late August and early September, and two new groups were made up.

One cow in each management system, used for the digestibility-intake study *(2)*, was maintained solely on pasture during the first part of the 1954 season. The remaining animals in both trials were fed only a concentrate ration according to Morrison's recommendations for cows on good pasture *(11)*.

In 1953, the cows were weighed at weekly intervals prior to evening milking, but these data were found to be of limited value in determining weight changes. Accordingly, in 1954, the cows were weighed every other day prior to leaving the barn after morning milking. Changes in weight due to water fill were considered to be minimum at this time.

Milk yield was recorded twice daily and samples were taken from an evening and morning milking every 10 days for butterfat analysis. Milk production was converted to fat-corrected milk (FCM) *(4)* for computation of the TDN requirements for production.

TDN production from pasture was determined according to the difference method of Knott *et al. (10)*. The average body weight during each unbroken period on pasture was used to estimate the TDN requirement for maintenance during that period. The difference between the beginning and ending weight was used as an estimate of weight gain or loss during a particular period. The averages of the first and last two weights were used in 1954. Gains and losses in weight were considered separately in the calculations due to differences in TDN allowances for these changes.

The TDN supplied in the concentrate portion of the ration was estimated according to Morrison's tables *(11)* and according to the method of estimating TDN from proximate composition analysis developed by Schneider *et al. (15)*.

RESULTS AND DISCUSSION

Summaries of animal and pasture production during the two trials are presented in Table 2. Although very similar, TDN production during both seasons was slightly higher under the strip grazing system.

The average daily production of FCM by the different groups is depicted graphically in Figures 2 and 3. The groups under the rotational system of man-

TABLE 2

Production from bromegrass pasture grazed under two management systems

	Rotational grazing	Strip grazing	Rotational grazing	Strip grazing
	1953 trial		*1954 trial*	
Number of cows	4	4[a]	4	4
Acres	2.1	2.1	3.2	3.2
Days on pasture	55	57	76	76
Average daily production per cow				
Fat-corrected milk *(lb.)*	27.62	26.22	29.37	32.63
Weight gain *(lb.)*	1.52	1.71	0.66	0.99
Weight loss *(lb.)*	0.15	0.25	0.23	0.39
Average daily grain ration per cow *(lb.)*	7.47	7.18	5.63	6.43
Production per acre from pasture[b]				
Total digestible nutrients	1860	1941	1532	1624
Standard cow days	116	121	96	101

[a] Substitution made 8-4-53 because of acute mastitis.
[b] Calculated according to the method of Knott *et al. (10)*.

agement show a cyclic pattern in their lactation curves, which appears to be related to the rotation from paddock to paddock. The level of production fell during the last few days on the previously grazed paddock and was at a low level as grazing of the new paddock was initiated. Production rose to a maximum when the paddock was approximately half depleted and fell off sharply as the remainder of the herbage was utilized.

Milk production under the strip grazing system was related to the intensity of grazing. Production tended to fall off as grazing intensity was increased beyond a certain level and recovered as the intensity was reduced. Further ramification of these interactions will be discussed in the subsequent papers on the digestibility-intake studies and animal behavior.

Differences in FCM production were analyzed statistically according to the methods of Goulden *(5)* by using paired cows as replicates and periods on individual paddocks as sub-plots. Highly significant differences were observed be-

TABLE 3

Average weights of the two groups of cows during the 1953 trial

Date	Rotational grazing	Strip grazing
6-2-53	1088	1056
6-8-53	1066	1080
6-23-53	1130	1082
7-1-53	1139	1112
7-7-53	1112	1125
7-16-53	1116	1143
7-23-53	1176	1163
7-28-53	1159	1176
8-4-53	1170	1110
8-11-53	1139[a]	1138
8-18-53	1090	1153
8-22-53	1112	1179
8-25-53	1101	1167
9-9-53	1103	1124

[a] Substitution made 8-4-53.

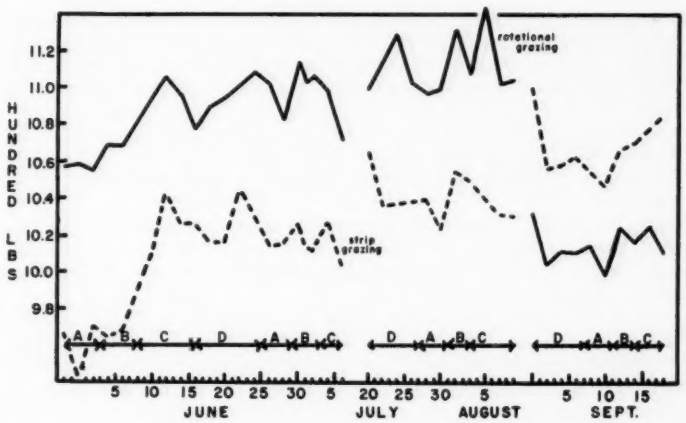

FIG. 4. Average bi-daily live weights, 1954 trial. Solid line—rotational, broken line—strip grazing. Arrows indicate periods when the rotational group was on specific paddocks. Breaks indicate periods when the groups were removed from the pasture. New groups were used during the third grazing cycle.

tween periods, which may be attributed mainly to the normal decline in production with advancing lactation. Differences in production due to management were not significant.

The average live weights of the two groups at different times during 1953 are listed in Table 3. The average bi-daily weights during 1954 are illustrated in Figure 4. Weight changes did not appear to be specifically related to system of management.

SUMMARY AND CONCLUSIONS

Two efficient systems of grazing management were compared during 1953 and 1954. A practical system of utilizing electric fencing for strip grazing has been described.

Total utilization of a single species grass pasture *(Bromus inermis)* at a moderate level of production was only slightly more efficient when strip grazing was employed than when conventional rotational grazing was used. Nearly all the potential production from a pasture of this type was utilized under a good system of rotational grazing.

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THE UTILIZATION OF SMOOTH BROMEGRASS *(BROMUS INERMIS)* UNDER ROTATIONAL AND STRIP GRAZING SYSTEMS OF PASTURE MANAGEMENT. II. DIGESTIBILITY-INTAKE STUDIES[1, 2]

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Several naturally occurring and artificial indicators have been proposed to circumvent the total collection of feces in digestion trials *(4)*. The use of plant pigments as naturally occurring indicators in digestibility studies was first proposed by Reid *et al. (8)*. In this procedure forages and feces were extracted in 85% acetone, and pigment concentrations were determined spectrophotometrically at 406 mμ. The ratio of plant to fecal pigments was used to compute indigestibility. In later studies *(7)*, formulas were derived which enabled the computation of either plant pigment concentration or digestibility of consumed herbage from fecal pigment concentrations. Use of either of these formulas precluded the manual sampling of herbage, thereby avoiding sampling error resulting from selective grazing.

Reid *et al. (7)* suggested the use of two indicators in the evaluation of pasture by grazing animals. A naturally occurring indicator, plant pigment, would be used to estimate digestibility and an added indicator, chromium oxide, would be used to estimate total fecal output. From these data, total intake from the pasture could be computed. They also suggested the use of "grab samples" to circumvent the necessity of total fecal collections. Smith and Reid *(11)* have demonstrated that chromium oxide could be successfully administered in either gelatin capsules or as a portion of the concentrate ration.

The pigments involved in these procedures have been studied by Irvin *et al. (3)* and Smart *et al. (10)*. The chemical nature of the pigments was altered during passage through the digestive tract, but these changes had little apparent effect on the light absorption properties of the mixture of pigments at 406 mμ. Smart *et al.* cautioned, however, that the digestibility procedure appeared to be limited to diets containing sufficient amounts of the chloroplast pigments.

Until the development of these techniques, pasture evaluation has depended upon indirect measurements. Although useful in pasture experimentation, the latter have been inadequate in revealing the day-to-day interaction between the pasture and the grazing animal. This digestibility-intake study was incorporated into a pasture management study to investigate the practical application of the chromogen–chromium oxide ratio technique.

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[2] Data presented are from a thesis submitted by A. L. Brundage to the Graduate Faculty of the University of Minnesota in partial fulfillment of the requirements for a Doctor of Philosophy degree.

EXPERIMENTAL PPROCEDURE

Descriptions of the pastures and the management systems employed have been given previously *(1)*. The chromogen–chromium oxide technique suggested by Reid was used during the first two grazing periods of the 1954 trial (May 29 to July 6 and July 20 to August 9) to obtain an estimate of the dry matter intake and apparent dry matter digestibility under the two systems of management (rotational and strip grazing). One animal in each group of four was maintained solely on pasture during this time. The digestibility-intake studies were run on these two animals.

Chromium oxide was employed as the added indicator. Each animal was given 10 g. daily in a 1-oz. gelatin capsule at 7:30 A.M. As the chromium oxide only partially filled these capsules, the total specific gravity was less than that of the rumen contents. It was postulated that these capsules would float to the posterior of the rumen, as a result of normal rumen activity, prior to releasing their contents, facilitating a more thorough mixing of the inert substance with the rumen mass. Ten grams of chromium oxide can be placed in a ½-oz. capsule. It was postulated that such a capsule, possessing a high specific gravity, would sink rapidly into the anterio-ventral portion of the rumen, limiting the complete mixing of the indicator with the rumen mass.

A serious problem resulted from the use of lighter capsules. If the animal began to actively ruminate immediately after administration, the capsules were sometimes drawn up with the regurgitated bolus, broken, and expectorated. This occurred several rumen cycles after the capsule was given.

Fecal samples were collected as rectal grab samples at 7:00 A.M. and 4:00 P.M. These were stored individually under refrigeration. As the experiment progressed, they were composited on an equal wet-weight basis into 3-day consecutive samples. Each composited sample contained 2 days' feces in common with the sample immediately preceding and following it, and one day's feces in common with the samples preceding and following those. This resulted in a series of 3-day fecal samples that were essentially running averages of the daily fecal output while on pasture. A portion of each sample was preserved with three drops of toluene and placed under refrigeration. The remainder of each composited sample was placed in an aluminum plate and dehydrated in a forced air drying chamber at approximately 140° F. The sample was then ground in a Wiley mill through a medium fine screen and stored for chromium oxide analysis.

The dry fecal samples were analyzed for chromium oxide according to the method of Gherke *et al. (2)*. An iodometric technique was used to determine the concentration of chromate after oxidation *(9, 13)*.

The total fecal output was calculated from the following formula:

$$\text{Total fecal output} = \frac{\text{Chromium oxide ingested daily}}{\%\ \text{chromium oxide in feces (D.M.)}} \times 100$$

The average recovery of chromium oxide when it was analyzed as a reagent was 99.6%, and accordingly the daily intake of chromium oxide was corrected to 9.96 g.

The refrigerated feces were analyzed for chromogen concentration according to the method of Reid *et al. (8)* with a Beckman model B spectrophotometer.

In order to use Reid's formula for determining plant chromogen concentration from fecal chromogen, it is necessary that the chromogen units obtained from an instrument be equivalent to the chromogen units obtained with Reid's instrument at Cornell University *(7)*. The following procedure was used for this standardization. An indeterminate amount of fresh pasture herbage and feces from cows on pasture was extracted in 85% acetone-water solution with a Waring blendor. This extract was diluted until a light transmission of from 1 to 10% was obtained, and a portion was forwarded to Cornell in a refrigerated carton. The remainder was subdiluted in a series of dilutions, and transmission readings were obtained on the Alaska instrument. The unit concentration of the original solution, which was supplied by Reid from his instrument, was substituted into this series of subdilutions, and a regression equation was computed for estimating chromogen concentration from light transmission values on the Alaska instrument. This formula was:

$$\text{Units of chromogen per 100 ml. of extract} = 57.5606 - 28.7145X \pm 0.222$$

where X = the logarithm of the per cent transmission. By using this equation the chromogen concentrations determined with the Alaska instrument were found to be equivalent to those determined with the Cornell instrument.

A formula developed by Reid *et al. (7)* was used to calculate the chromogen concentration of the forage from the chromogen concentration of the feces. These two values were used to compute apparent dry matter digestibility by means of the following formula:

$$\text{Per cent apparent D.M. digestibility} = 100 - 100 \times \frac{\text{units chromogen/g forage (D.M.)}}{\text{units chromogen/g. feces (D.M.)}}$$

Dry matter content of the fecal samples was determined by drying in an oven at 100° C. Distilled water was mixed with the samples prior to drying to facilitate more uniform drying.

A multiple correlation analysis was computed according to the methods of Wallace and Snedecor *(16)* and Snedecor *(12)* to determine the relative influence of time, apparent dry matter digestibility, fecal output, total dry matter intake, and live weight on daily FCM production. Daily weights were obtained by halving the differences between the bi-daily weights.

RESULTS AND DISCUSSION

The previous work reported on the use of indicators, both naturally occurring and artificial, has been directed toward development and evaluation of procedures or toward the static determination of mean apparent dry matter digestibility and total dry matter intake on a relatively large area for a period of several days. In such studies, good agreement often has been obtained between procedures involving the use of indicators and conventional total collection trials. When the herbage has been clipped and fed in inside trials, agreement has been improved by the fact that intake could be maintained at a constant level, thereby

reducing the daily variation in excretion of feces. In both types of trials, several days' fecal collections are composited for analysis to reduce the fluctuations in daily fecal output.

The interactions between a pasture and the grazing animal are not static ones, which may be revealed completely by measurements taken at weekly or greater intervals, regardless of how accurate the measurements. The fecal sampling and compositing procedure described has been designed to give an estimate of the daily dry matter intake and digestibility of herbage consumed by a grazing animal without sacrificing the reduction in error gained by analyzing samples composited over 3-day periods.

The daily FCM production, dry matter intake, and apparent dry matter digestibility for the two "test" cows used during the first portion of the 1954 trial are illustrated in Figure 1. The simple correlation coefficients and variances for time, digestibility, fecal output, total dry matter intake, live weight, and milk production are recorded in Table 1. The negative correlation between

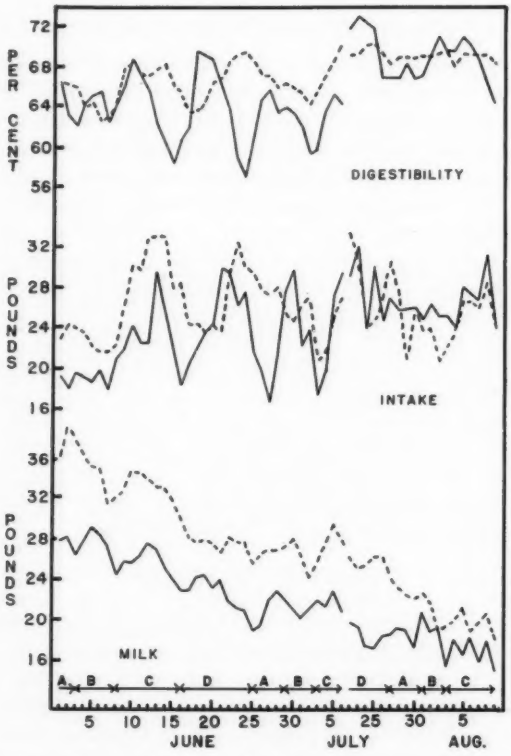

FIG. 1. Daily FCM production, dry matter intake, and apparent digestibility. Solid lines—cow on rotational grazing, broken lines—cow on strip grazing. Arrows indicate dates on specific rotation paddocks.

TABLE 1

Correlation coefficients and variances for the digestibility-intake phase of the 1954 trial

	Time	Digestibility	Fecal output	Total D.M. intake	Live weight	FCM
Cow under rotational grazing						
Time	13860	0.4328[b]	0.2179	0.5337[b]	0.7946[b]	−0.9267[b]
Digestibility		784.33	−0.3555[b]	0.3402[a]	0.1952	−0.3535[b]
Fecal output			21.81	0.7533[b]	0.4161[b]	−0.2166
Total dry matter intake				173.13	0.5597[b]	−0.4812[b]
Live weight					56715	−0.7567[b]
FCM						775.44
Cow under strip grazing						
Time	13860	0.6481[b]	−0.3629[b]	−0.0641	0.7058[b]	−0.9421[b]
Digestibility		221.07	−0.0905	0.3522[b]	0.6444[b]	−0 5069[b]
Fecal output			12.60	0.8989[b]	0.0368	0.3613[b]
Total dry matter intake				137.72	0.3061[a]	0.1263
Live weight					49370	−0.6963[b]
FCM						1,525.44
	Degrees of freedom = 53					

[a] Significant at the 5% level.
[b] Significant at the 1% level.

time and milk production was very high for both cows. A large portion of this correlation is probably associated with the normal decline in level of production with advancing lactation. The variances for the cow under the strip grazing system are lower for all functions except milk production, which indicates that these were less variable for this cow than for the cow under the rotational system of grazing. The higher variance for milk production may be explained by the greater decline in production over the season, for this cow was less advanced in her lactation at the start of the experiment.

In Table 2 is presented the statistical analysis of the multiple correlation coefficients. In these data total dry matter intake was related mathematically, as well as biologically, to fecal output and digestibility. The initial difficulty in the correlation analysis was the determination of the correct pairing of fecal samples with production and live weights. It was assumed that the analytical value of any fecal sample represented a mean value equivalent to the fecal output on the middle day of the 3 days that made up the sample.

The analysis was computed initially with production paired with the previous day's fecal output, assuming that there was a lag in time between fecal production and physiological utilization of the digested nutrients for synthesis of milk. A highly significant partial correlation (+0.3997) was obtained between milk production and digestibility for the strip-grazed cow when time was held constant. The data for the rotationally grazed cow showed a highly significant partial correlation (−0.3578) between fecal output and production, and a nonsignificant partial correlation between digestibility and milk production when time was held constant.

This negative correlation was difficult to explain biologically with the available data. For this reason, milk production was paired with fecal output on the same day. There was an increase in the multiple correlation coefficient for production, digestibility, and time with the data from the strip-grazed cow. The

TABLE 2

Statistical analysis of multiple correlation coefficients

Source of variation	Degrees of freedom	Mean square	F
Cow under rotational grazing			
Regression (time and production)	1	665.95	322.34[a]
Error of estimate (time & prod.)	53	2.066	
Error of estimate (time, prod., & dig.)	52	2.065	
Error control	1	2.09	1.01
Error of estimate (time, prod., & fecal out.)	52	2.101	
Error control	1	0.23	0.11
Error of estimate (time, prod., & total dry matter intake)	52	2.103	
Error control	1	0.15	0.07
Error of estimate (time, prod., & weight)	52	2.091	
Error control	1	0.77	0.37
Cow under strip grazing			
Regression (time and production)	1	1353.98	418.54[a]
Error of estimate (time & prod.)	53	3.235	
Error of estimate (time, prod., & dig.)	52	2.755	
Error control	1	28.22	10.24[a]
Error of estimate (time, prod., & fecal out.)	52	3.286	
Error control	1	0.61	0.19
Error of estimate (time, prod., & total dry matter intake)	52	3.171	
Error control	1	6.56	2.07
Error of estimate (time, prod., & weight)	52	3.233	
Error control	1	3.36	1.00

[a] Significant at the 1% level.

partial correlation coefficient between digestibility and production, independent of time, was changed slightly (+0.4062). The partial correlation coefficient between fecal output and production, independent of time for the rotationally grazed cow, was reduced to a nonsignificant value (−0.0402).

The data were then shifted so that fecal production occurred the day after milk production. The correlation between digestibility and milk production independent of time for the strip-grazed cow was reduced to +0.1934. The partial correlation coefficient between fecal output and milk production for the rotationally-grazed cow was slightly altered (+0.061).

From these comparisons it was concluded that milk production occurred on the same day as fecal production, and the correlation analysis was set up accordingly. This is in agreement with the data of Keener *et al.* (*5*), which showed that the peak of excretion of radioactive sulfur in milk and feces was on the second day after administration.

The regression of milk production on time was highly significant under both management systems. Multiple correlation coefficients were computed and tested for significance by adding one of the other variables to time and testing the significance of the error control obtained over the simple correlation between time and production. With the cow on the strip grazing system, a highly significant increase in error control was obtained when digestibility was added to time. The simple correlation between time and production was −0.9421. The

multiple correlation between time, digestibility, and production was −0.9519. The partial correlation between digestibility and milk production with time constant was +0.4062.

This may be explained on the basis of a dynamic interaction between intake and available herbage, assuming that the cow attempts to maintain a uniform daily intake regardless of the amount or quality of herbage offered. This has been reported by Waite *et al. (14, 15)*, who found that intake was remarkably constant at 24 to 26 lb. a day for close folded cows. The intake varied from 22 to 33 lb. in the trial reported here. If the herbage offered on any one day is limited, the animal must eat a higher percentage of it in order to fulfill her hunger requirements. When this happens, the digestibility of the consumed herbage may be reduced, as it was in the early part of this trial. Milk production appeared to be depressed by consumption of this material of lower nutritive value.

When an over abundance of herbage is offered, the animal can fulfill her hunger requirements from a smaller percentage of the total. Under these conditions, the quality of the consumed herbage will be higher than the average quality of the available herbage because of animal selection. Thus, from June 17 through June 24 the digestibility of the consumed herbage steadily increased in spite of the fact that the available material was at its most mature state during this time. In conjunction with this, milk production remained relatively constant after a period of sharp decline.

McCullough *et al. (6)* reported that the correlation between digestibility and weight gain did not become significant until digestibility had declined below a certain level. When this is considered, in addition to the above observations, the importance of providing sufficient daily herbage is emphasized under the strip grazing system of management, so that the animals need eat only a certain percentage to satisfy their hunger requiremnts. Additional efficiency of utilization is obtained at the expense of lowering the nutritive value of the consumed herbage.

Time was the only variable which was correlated significantly with milk production under the rotational system of management. Although the simple correlation coefficients of some of the other variables with milk production were significant, this appeared to be due to their correlation with time, for none of the multiple correlation coefficients resulted in a significant error control over the simple correlation between time and production.

The data for the cow under the rotational system cannot be considered in their entirety, as the data for the other cow were, for her grazing was divided into separate periods by the management system. The data for each of these paddocks, although insufficient for definite conclusions, suggest relationships between total dry matter intake, digestibility, and daily milk production. As is shown in Figure 1, production tends to be low the first few days on a paddock, rises to a maximum when the paddock has been partially utilized, and falls sharply during the last few days. Digestibility and total dry matter intake follow a similar pattern. Two factors tended to obscure this relationship. First,

the time on many of the paddocks was too short for this pattern to be clearly differentiated before the cows were shifted to a new paddock. Secondly, the animals were continually shifting between lush, highly palatable herbage, and defoliated material of low palatability, and this may have altered the pattern of excretion of the indicators.

During the 1954 trial three clippings were taken from each paddock prior to grazing to obtain an estimate of available herbage. These data are compared in Table 3 with the estimated intakes of the test cows and the calculated TDN intakes of the four groups over the entire grazing period. The average daily gains or losses during each major grazing period were used to calculate the TDN requirement for weight changes on each paddock. This helped compensate for error due to differences in fill at the beginning and end of each paddock. The intake of the test cows was multiplied by four to give estimates of the intakes of

TABLE 3

Comparison of the estimates of pasture production made from samples clipped prior to entry, from the chromogen-chromium oxide ratio technique, and from calculated TDN requirements

Paddock	Days grazed	Clipping	Ratio (1 cow)	Ratio × 4	Calculated
Rotational grazing		*(lb. D.M.)*	*(lb. D.M.)*	*(lb. D.M.)*	*(lb. TDN)*
A	5	844.8	94.8	379.2	379.1
B	5	337.6	96.6	386.4	367.2
C	8	588.0	188.7	754.8	565.6
D	9	849.6[a]	224.1	896.4	634.5
A	4	271.2	85.4	341.6	290.3
B	4	271.2	93.1	372.4	288.2
C	3	82.4	76.3	305.2	217.3
D	7	469.6	194.7	778.8	393.0
A	4	328.8	102.3	409.2	221.3
B	3	350.4	76.4	305.6	165.5
C	6	570.4	161.0	644.0	328.8
D	7	812.8			412.9
A	4	436.8			233.4
B	3	343.2			174.0
C	4	381.6			231.0
Strip grazing					
A	5	714.4	142.2	568.8	425.3
B	5	302.4	111.1	444.4	408.8
C	8	720.0	241.5	966.0	649.5
D	9	886.4[a]	241.6	966.4	715.4
A	4	260.8	108.3	433.2	331.4
B	4	174.4	98.7	394.8	330.7
C	3	172.8	78.2	312.8	249.8
D	7	658.4	196.4	785.6	340.8
A	4	328.8	97.2	388.8	189.6
B	3	232.0	66.5	266.0	133.5
C	6	663.2	154.1	616.4	268.8
D	7	636.8			463.3
A	4	674.4			264.0
B	3	290.4			186.0
C	4	712.8			238.8

[a] Clippings taken before and after grazing.

the four cows. These data are included for purposes of comparison and not as precise measurements of the intakes of the entire group.

TABLE 4

Correlations for the comparisons of clipping, ratio technique, and calculated TDN requirements

Variables	Correlation coefficients	
	Rotational grazing	*Strip grazing*
Yields from clippings and calculated TDN	0.7127[b]	0.5935[a]
Yields from clippings and yield estimated from the ratio technique	0.6355[a]	0.9000[b]
Calculated TDN and yields estimated from the ratio technique	0.7572[b]	0.8539[b]

[a] Significant at the 5% level.

[b] Significant at the 1% level.

The correlation coefficients for these estimates are presented in Table 4. The coefficients are comparable for the rotational system of managment. Under the strip grazing system, the yields estimated from clippings and calculated TDN requirements are less closely related to each other than they are individually related to the yield estimated by the ratio technique.

The digestibility-intake data were obtained from only one animal in each system. For this reason, these data do not show the degree of individual variation to be expected under these conditions.

The estimates of pasture utilization calculated from clippings and the TDN requirements of grazing animals are useful in determining the seasonal production of pastures. Their usefulness in determining pasture yields for short periods during the season is limited because they are not precise enough to account for the daily variations in the grazing animal, the pasture, and their interaction.

SUMMARY AND CONCLUSIONS

The combined chromium oxide–chromogen technique has been employed to determine the apparent dry matter digestibility and intake of two milking dairy cows grazing smooth bromegrass under rotational and strip grazing systems of pasture management. A fecal sampling and compositing procedure has been described whereby estimates of daily dry matter intake and apparent dry matter digestibility may be obtained without sacrificing the reduction in error gained by analyzing samples composited over 3-day periods. Statistical analysis of the data has been presented which supports the conclusion that fecal production and milk production, resulting from previous ingestion of pasture herbage, occur simultaneously. A positive correlation between milk production and apparent dry matter digestibility independent of time has been demonstrated under the strip grazing system of management. Estimates of pasture utilization based on digestibility-intake studies, calculated TDN requirements, and clippings made prior to grazing the paddocks were compared. All three estimates of pasture utilization have been shown to have valid applications.

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THE UTILIZATION OF SMOOTH BROMEGRASS *(BROMUS INERMIS)* UNDER ROTATIONAL AND STRIP GRAZING SYSTEMS OF PASTURE MANAGEMENT. III. ANIMAL BEHAVIOR[1,2]

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The systematic study of animal activity was initiated by Cory *(4)* with a detailed analysis of the activities of cattle on the open range. Tribe and Gordon *(8)* have divided behavior studies into three phases. The first is the anecdotal period, when attempts are made to explain animal behavior on the basis of human emotions. The second, they call the experimental period, when cows are intensively observed in restricted and unnatural environments. The third phase is the modern period when the cows are observed in their natural environment with careful attention being given to the explanation of particular behavior patterns.

Extensive reviews of animal behavior investigations have been presented by Tribe *(7)* and Hancock *(5)*. Both reviewers stressed the importance of the adaptability of the grazing animal. Animal production was considered to be the best measure of the value of a pasture or management system. Behavior studies were indicated for their possible usefulness in explaining some variations in production observed. Their place in pasture investigation was summarized by Tribe: "Without making these continuous observations, it would be impossible to appreciate, as we must, the implications of the animals' grazing behavior, but it is these implications that are the reward of the watcher, not the factual data that during a paricular day in a particular locality one particular animal spent a particular time grazing, resting, or idling."

Behavior studies were included in the pasture management experiment to determine the effect, if any, of the particular management systems on animal activity and the implications of this activity as regards the pasture-animal complex.

EXPERIMENTAL PROCEDURE

Descriptions of the pastures and the management systems employed have been given previously *(3)*. Nine behavior observations were conducted during the 1953 season. The cows were continuously under observation from the time they entered the pasture after morning milking until they were removed for evening milking (a period of approximately 6 hours). During this time the activity of each was arbitrarily placed in one of the following categories at

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[1] Alaska Agricultural Experiment Station Technical Publication 9.

[2] Data presented are from a thesis submitted by A. L. Brundage to the Graduate Faculty of the University of Minnesota in partial fulfillment of the requirements for a Doctor of Philosophy degree.

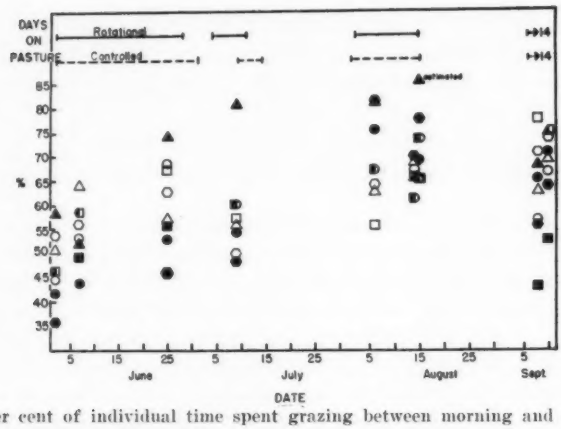

FIG. 1. Per cent of individual time spent grazing between morning and evening milking. Solid—rotational, open—strip grazing.

5-minute intervals: grazing, standing and ruminating, standing and loafing, lying and ruminating, and lying and loafing. These data were presented previously at the 46th annual meeting of the American Society of Animal Production *(1)*.

RESULTS AND DISCUSSION

Although the original data were for individual animals, herein they have been consolidated for the two management groups for ease in presentation and discussion. In addition, the data presented will be limited to grazing time and pattern. Inasmuch as the observations were of limited duration (6 hours), all the other activities tabulated were directly influenced by grazing time.

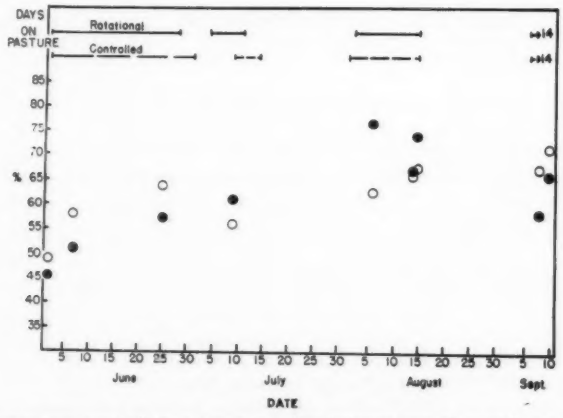

FIG. 2. Average per cent of time spent grazing between morning and evening milking for two groups of four cows each. Solid—rotational, open—strip grazing.

The individuality of grazing is illustrated in Figure 1, which depicts the grazing time for individuals. The grazing time of an animal relative to that of others tends to remain the same between observations.

Figure 2 illustrates how the groups adjusted their grazing habits to changes in the pastures. Both groups steadily increased their total grazing time during the day as the season advanced. Initially, this was related to selection imposed by the increasing maturity of the first growth of herbage with the resultant decrease in total nutritive value. In spite of this decline, the apparent dry matter digestibility of herbage consumed by the cow under the strip grazing system steadily increased during this period of the 1954 trial *(2)*. These apparently contradictory observations support the conclusion that this animal was actively selecting her diet at this time. The increases in grazing time observed support the conclusion that the selection of a good quality diet from mature pasture herbage requires more time than from immature herbage.

Subsequently, quality and quantity both are factors that influence grazing time later in the season when the cows are regrazing the pasture. Jones *(6)* suggested that the growth after winter rest was more palatable than subsequent

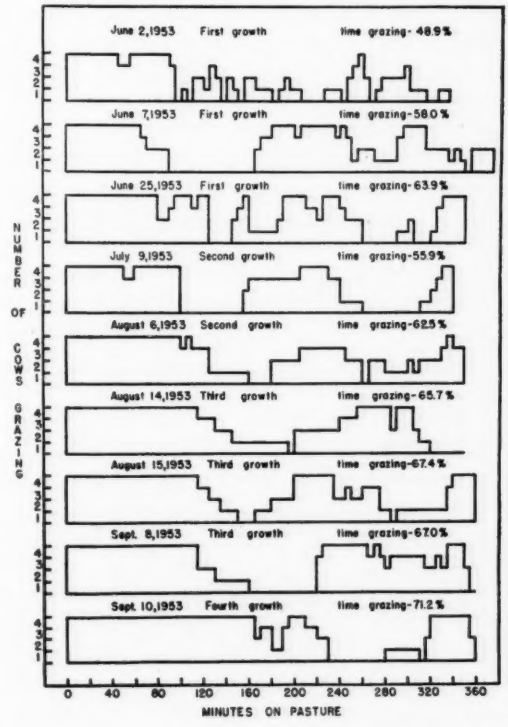
June 2,1953 First growth time grazing- 48.9%
June 7,1953 First growth time grazing-58.0%
June 25,1953 First growth time grazing-63.9%
July 9,1953 Second growth time grazing-55.9%
August 6,1953 Second growth time grazing-62.5%
August 14,1953 Third growth time grazing-65.7%
August 15,1953 Third growth time grazing-67.4%
Sept. 8,1953 Third growth time grazing-67.0%
Sept. 10,1953 Fourth growth time grazing-71.2%
NUMBER OF COWS GRAZING
0 40 80 120 160 200 240 280 320 360
MINUTES ON PASTURE

FIG. 3. Grazing patterns between morning and evening milking of a group of four cows on strip grazing.

growth, even though the latter might be of equal leafiness. In addition to this, selective grazing is employed to avoid consumption of material soiled by dung and urine. The grazing time is also increased as a result of the physical nature or quantity of the herbage. When subsequent growth is sparse, the quantity consumed in any one bite is reduced, increasing the number of bites required per unit of consumption.

The distribution of the grazing time for the strip grazed group (Figure 3) appeared to be influenced by advancing season also. The initial period of grazing was steadily lengthened as the season progressed. Grazing activity was definite during this period, and aimless walking was kept at a minimum. The fact that initial grazing time during observation 4, when they were on lush second growth, was comparable to the initial times during observations 1 and 2, when they were on lush first growth, suggests that its length is determined by the satisfaction of the hunger urge, for initial time was increased on material of poor quality and on material of reduced quantity and quality. Postponement of hunger satisfaction might result from the intake of material of lower nutri-

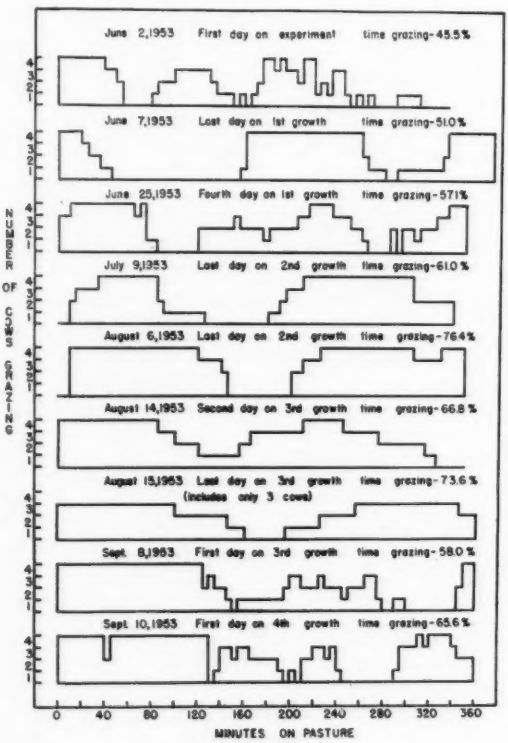
June 2,1953 First day on experiment time grazing-45.5%
June 7,1953 Last day on 1st growth time grazing-51.0%
June 25,1953 Fourth day on 1st growth time grazing-57.1%
July 9,1953 Last day on 2nd growth time grazing-61.0%
August 6,1953 Last day on 2nd growth time grazing-76.4%
August 14,1953 Second day on 3rd growth time grazing-66.8%
August 15,1953 Last day on 3rd growth time grazing-73.6%
(includes only 3 cows)
Sept. 8,1953 First day on 3rd growth time grazing-58.0%
Sept. 10,1953 First day on 4th growth time grazing-65.6%
NUMBER OF COWS GRAZING
0 40 80 120 160 200 240 280 320 360
MINUTES ON PASTURE

FIG. 4. Grazing patterns between morning and evening milking of a group of four cows on rotational grazing.

tive value, lowered intake per unit of time as a result of reduction in quality and quantity, or an interaction of the two. Grazing was always more sporadic in the afternoon. Often, one or more animals would be engaged in other activities while their mates were grazing. This suggests that the lengthening of the initial grazing period was an adequate adjustment enabling the grazing cows to satisfy their major needs during the morning.

Data are unavailable to show how these animals responded to the depleted strip when they returned to the pasture after the evening milking.

The effect of advancing season upon the distribution of grazing time (Figure 4) for cows that grazed rotationally was modified by the degree of defoliation a paddock had undergone prior to the observation. When the animals were grazing an area which had undergone little or no defoliation previously, the grazing patterns were similar to those observed with the strip-grazed group. This suggests that a comparatively effective and lasting degree of satisfaction was obtained during the initial grazing period.

When defoliation of an area was nearly complete, grazing during the morning appeared to be conducted under protest. On two occasions the animals refused to graze when they entered the pasture. Instead, they gathered at the fence separating the two groups and complained audibly for several minutes. When they did graze, they were very restless and failed to graze in any one area for more than a few minutes before moving on to completely new territory. The grazing during the afternoon was more compulsory, as the animals reduced their restless wandering and concentrated on grazing the remaining herbage.

These observations suggest that the animals failed to receive any lasting degree of satisfaction from their morning's grazing activity and were forced to graze actively during the afternoon to make up this deficit. The average lactation curves indicate that nutrient intake under these grazing conditions was inadequate, for daily production declined sharply during the last few days on a paddock *(3)*. Total dry matter intake and digestibility also decreased during this time *(2)*. Thus, the responses of the animals to this environment were inadequate. Their recovery was dependent upon a change in environment, in this case movement to a new paddock, rather than adaptation.

The grazing behavior of the cow whose time was estimated (Figure 1, observation 7) was unique. Her entire morning was spent listlessly standing, or lying down. Absolutely no interest was expressed in grazing, and very little walking was done. There was no rumination during the entire observation. Her grazing time was 36.1% of the total time on pasture, and this was done during late afternoon. It was discovered during the evening milking that she was suffering from acute mastitis. This had not been observed during the morning milking.

This is an example of the effect of illness upon a grazing cow. In addition to being disturbed by the illness itself, her physiological balance is further impaired by a reduction in nutrient intake. It emphasizes the importance of providing conditions whereby nutrient intake will be enhanced in addition to treating the illness directly. In the above instance there was no indication that she derived

any benefit from being on pasture. Being there may have actually complicated the illness.

SUMMARY AND CONCLUSIONS

Nine continuous behavior observations have been conducted on two groups of dairy cows grazing smooth bromegrass under rotational and strip grazing systems of management. The total grazing time of both groups increased with advancing season. This has been attributed to increasing plant maturity during initial grazing and to soiling and reduced available herbage during subsequent grazing. Advancing season also resulted in an increase in initial grazing time for the group under the strip grazing system. This was apparently satisfactory compensation for reduced palatability and availability, for afternoon grazing activity remained relatively constant over the experiment. The pattern of grazing activity for the rotationally grazed animals was influenced by the degree of previous defoliation of a paddock prior to the observation in addition to advancing season. When grazing a nearly depleted area, the period of intense grazing activity was shifted to the afternoon.

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INFLUENCE OF CHLORTETRACYCLINE ON IN VITRO CELLULOSE DIGESTION BY BOVINE RUMEN MICROORGANISMS[1,2]

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Numerous investigators have demonstrated that feeding of chlortetracycline (aureomycin) to young dairy calves stimulates growth. On the other hand, the limited data that have been reported with older dairy cattle suggest no marked beneficial or detrimental effects from feeding various levels of aureomycin *(7, 13)*. There are, however, some indications that antibiotics may influence rumen fermentation processes.

Bell *et al. (1)* found that feeding 0.2 g. of aureomycin per animal daily to steers decreased the apparent digestibility of crude fiber and dry matter. The most pronounced effect was on the digestibility of crude fiber, which suggested to the authors that the aureomycin depressed the activity of the cellulolytic microorganisms in the gastrointestinal tract. Chance *et al. (3)*, in studies with rumen-fistulated steers, reported that aureomycin at a level of 0.5 g. increased the number of bacteria and that higher levels of aureomycin inhibited some of the cellulose-digesting bacteria in the rumen. Horn *et al. (6)* observed that aureomycin depressed the digestibility of protein and crude fibers by steers to a greater extent than did penicillin and that the two antibiotics decreased nitrogen retention to about the same extent. Using an "artificial rumen," Kesler *(9)* found that rumen microorganisms from terramycin-fed calves digested less cellulose (24.2%) than those from animals fed no antibiotic (77.4%). Wasserman *et al. (17)*, employing the artificial rumen, found that cellulolytic activity was stimulated by low concentrations of penicillin and streptomycin and by all concentrations of neomycin studied, whereas comparatively high concentrations of streptomycin and chloromycetin inhibited cellulose digestion.

Since cellulose plays such an important role in the nutrition of ruminants, it seemed desirable to study further the effect of feeding aureomycin on cellulose digestion in the rumen. In the present investigation the artificial rumen technique was employed to *(a)* estimate the efficiency of cellulose digestion by rumen microorganisms in calves and in lactating dairy cows fed aureomycin over an extended period of time, *(b)* determine the effect of the addition of aureomycin

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to the artificial rumen medium, and *(c)* study the effect of aureomycin feeding on the types and numbers of rumen microorganisms.

EXPERIMENTAL PROCEDURE

Twenty lactating cows and four young calves approximately 4 months of age were used in this experiment. The animals were of the Ayrshire, Brown Swiss, Guernsey, Holstein, and Jersey breeds, selected from the Iowa State College herd. The cows were divided into two groups of ten animals each, according to breed and previous treatment. All cows in one group had received aureomycin from an early age. The calves were paired on the basis of previous treatment and age, and one of each pair had received aureomycin since 4 days of age.

The cows received a concentrate mixture which contained ground shelled corn, 400 parts; crushed oats, 500 parts; wheat bran, 200 parts; linseed oil meal, 100 parts; soybean oil meal, 200 parts; steamed bone meal, 28 parts; and salt, 14 parts. The concentrate mixture was fed according to production, approximately 1 lb. to each 4 lb. of milk. Each cow also received 8-12 lb. of alfalfa hay and 30-50 lb. of corn silage daily, fed in two equal feedings. Aurofac 2A[5] was fed with the concentrate mixture in amounts to furnish approximately 240 mg. aureomycin per cow daily for the supplemented animals. Except when indicated otherwise, this supplement was fed at the afternoon feeding.

The calves received hay ad libitum and a maximum of 4 lb. of concentrate mixture per calf daily, fed in two equal feedings. The composition of the concentrate mixture was ground shelled corn, 400 parts; crushed oats, 400 parts; soybean oil meal, 200 parts; steamed bone meal, 18 parts; and salt, 9 parts. Aureomycin, fed in the form of Aurofac 2A, was mixed with the concentrate mixture to furnish approximately 80 mg. aureomycin daily.

Samples of rumen liquid were obtained at approximately the same time each day, between 7 and 9 A.M., with the aid of a stomach tube and a hand-operated stomach pump. For the cows, collection time except where indicated otherwise was about 16 hours, 4 hours, and 1 hour after feeding aureomycin, hay and concentrates, and silage, respectively.

Cellulose digestion was estimated by means of an artificial rumen technique *(5)*. Rumen microorganisms for use as inocula in the artificial rumens were obtained by centrifugation of rumen ingesta and were placed in a mineral-cellulose[6] medium. Cellulose digestion was measured by analysis of this medium in triplicate for cellulose before and after a 24-hour digestion period. Samples were obtained from each animal on three different days.

Observations of the extent of cellulose digestion also were made on samples taken from artificial rumens where various levels of aureomycin had been added to the fermentation tubes. The levels added were 2.4, 1.6, 0.8, 0.4, and 0 γ per

[5] Supplied by Lederle Laboratories Division, American Cyanamid Co., Pearl River, N. Y. This feeding supplement contained 3.6 g. of aureomycin per pound.

[6] Solka-floc, obtained from Brown Co., Berlin, N. H.

milliliter of fermentation mixture. These levels were chosen because calculations from the amount of antibiotic fed to the cows indicated the rumen should contain approximately 2.4 γ of aureomycin per milliliter of rumen liquid immediately after antibiotic feeding. (Admittedly, this estimate is subject to considerable error.) The lower levels were used because it was not known how much aureomycin was present in the rumen liquor at the time of collection, 16 hours after feeding.

A microbiological assay was carried out to determine the level of aureomycin in the rumen fluid. The liquid obtained by centrifugation of the original rumen material was employed for this assay with 1 ml. being serially diluted with an equal volume of methylene blue broth. The test organism was *B. cereus* No. 5 (Dornbush and Pelcak, *4)* which was used in a modification of the procedures of Schipper and Petersen *(14)* and Maddock *(10)*. In the present method the Wasserman tubes were replaced by 18 × 150 mm. screw cap test tubes (Kimble) which were carefully selected for equal size and optical properties. The inoculum was standardized to 85% T at 590 mμ, and 10 ml. of this inoculum were added to each tube. The tubes then were filled with a 1:50,000 methylene blue solution in Penassay broth (Difco). Precautions were taken during filling and sealing to avoid inclusion of air bubbles. After a 5-minute incubation to allow equilibration the tubes were read for initial transmission (Klett-Summerson photoelectric colorimeter). After a 2-hour incubation in a covered (for exclusion of light) water bath at 37.5° C., the tubes again were read for transmission. A standard curve for known amounts of aureomycin versus inhibition of methylene blue reduction was determined under similar conditions, with filtered rumen fluid containing no antibiotics being added as in the assay procedure.

The effect of aureomycin feeding on the numbers of various rumen microflora was estimated by employing five pairs of cows from the groups previously described. Rumen samples were withdrawn into vacuum bottles to reduce temperature change and were processed promptly. Nevertheless, marked between-sample variations occurred within animals, and therefore comparisons were based only on values obtained with pairs run simultaneously.

Samples of rumen ingesta from two aureomycin-fed cows and their paired controls were taken on two different occasions, and two aliquots from each sample were plated for standard counts on the following media:

1. Violet red bile agar (Difco) for coliforms.
2. Linden thioglycollate agar (Difco) for "total anaerobes" and with heated inocula (80° C. for 10 minutes) for clostridia.
3. Tryptone glucose beef extract agar (Difco) plus 1.5% Bacto-yeast extract for "total aerobes."
4. Littman oxgall agar (Difco) for fungi.
5. Chapman-Stone gelatin agar (Difco) without ammonium sulfate for total proteolytic organisms.
6. Lactobacillus medium, Rogosa *et al. (12)*.
7. Methyl cellulose medium *(16)* for cellulose digesters.
8. CP-3 *(11)* for glucose fermenters.

9. CP-3 medium, with lactate substituted for the glucose of CP-3, for lactate utilizers.

Rumen ingesta were collected in Thermos bottles and transported to the laboratory within a 2-hour period. Decimal dilutions from 10^{-2} to 10^{-8} were made in sterile distilled water blanks, and the plating operations were completed as quickly as possible after receipt of the samples. All plates were incubated at 37° C. for 48 hours, except for violet red bile agar plates, which were read at 24 hours.

Other important groups of rumen microorganisms undoubtedly exist for which differential culture techniques are not yet available or convenient. Such groups as the protozoa, spirochetes, and a large variety of symbiotic bacteria are not enumerated in the present study. In population studies not included in this report, thermophiles capable of growth on Tryptone glucose beef-extract yeast-extract agar at 60° C. and streptococci capable of growth on Mitis-Salivarius agar (Difco) at 37° C. were found to be very low in numbers, so they were not estimated in subsequent studies of rumen populations.

For some of the population studies each of the media given above was modified by the addition of 2.4 γ of aureomycin HCl per milliliter. On these antibiotic-containing media the depression in fermentative activity was greater than the inhibition of growth, compared to the results on media without antibiotic. Three additional pairs of animals (three aureomycin-fed cows and three controls) were used to study the effect of the addition of aureomycin to media on lactic acid utilizers, methyl cellulose utilizers, and glucose fermenters.

RESULTS AND DISCUSSION

The average cellulose digestion values during a 24-hour period where no aureomycin was added to the substrate are presented in Table 1. Aureomycin fed either to cows or to calves inhibited cellulose digestion by the rumen microorganisms. The mean values for cellulose digestion were approximately 12% higher for the nonsupplemented cows and 20% higher for the control calves than the means for the respective aureomycin-fed groups. An analysis of variance *(15)* showed the differences in cellulose digestion by microorganisms from the two groups of cows to be significant at $P = 0.01$. One week after the removal of aureomycin from the ration of the supplemented calves cellulose digestion

TABLE 1

Effect of aureomycin feeding on in vitro cellulose digestion

	No. of animals	Treatment	Trial No.			
			1	2	3	4
			(Cellulose digestion, %)			
Cows	10	Control	83.5	82.7	87.5	
	10	Aureomycin	70.2	73.8	74.5	
Calves	2	Control	77.5	76.6	81.4	73.9
	2	Aureomycin	62.6	55.0	57.0	82.3[a]

[a] One week after removal of aureomycin from ration.

TABLE 2
Effect of addition of aureomycin to substrate on in vitro cellulose digestion

Amount of aureo. added	No. of cows	Treatment of cows	Cellulose digested	
(γ/ml)			*(%)*	*(%)*
0	10	Control	86.5	
	10	Aureomycin		74.2
0.4	4	Control	25.5	
	4	Aureomycin		72.0
0.8	4	Control	24.4	
	4	Aureomycin		72.0
1.6	10	Control	11.6	
	10	Aureomycin		63.8
2.4	2	Control	9.2	
	2	Aureomycin		62.8

had increased markedly, but, since the number of animals was small, these data can be considered only as suggestive of possible adjustments occurring after removal of the antibiotic from the diet.

The effect on cellulose digestion of the addition of various levels of aureomycin to the fermentation tubes of the artificial rumen is shown in Table 2. The addition of aureomycin to the inoculum from the control cows had a marked depressing effect on the ability of the microorganisms to digest cellulose. Conversely, the addition of the same levels of aureomycin to the inoculum from the supplemented cows resulted in comparatively little change in cellulose digestion.

The results of microbiological assays of rumen samples for aureomycin concentration are presented in Table 3. These results indicate that aureomycin, when administered orally, is rapidly removed from the rumen liquid. Inactivation of aureomycin by rumen microorganisms has been observed by Jurtshuk *et al. (8)* in in vitro studies. In the amounts fed in the study reported herein, the aureomycin should be present to the extent of approximately 2 to 3 γ per milliliter of rumen liquor. The first analyses were run on samples of rumen liquid collected about 16 hours after the antibiotic was fed. The results from these analyses indicate that there is a very small amount of the antibiotic present at this time. Therefore, to reduce the time between feeding and sam-

TABLE 3
Aureomycin content of rumen fluid

No. of animals	Amount of aureo. fed daily	Time of aureo. feeding	Aureomycin
	(mg.)		*(γ/ml)*
6 cows	240	Daily allowance fed 16 hr. before sampling	0.077
2 cows	240	Daily allowance fed 4 hr. before sampling	0.225
2 calves	80	½ daily allowance fed 2 hr. before sampling	0.221

pling, aureomycin feeding of two cows was changed to the morning. No samples were collected from these cows for 1 week to allow adjustment to the new regime. Then rumen liquid samples from these two cows were collected approximately 4 hours after feeding the supplement. More aureomycin was present in these samples, but the values still were only about one-tenth the calculated amount based on estimated rumen capacity and assuming equal distribution and no loss. The fate of aureomycin after entering the rumen is not clear but the following hypotheses might be offered:

a) The antibiotic may be absorbed into the blood stream.

b) Part of the aureomycin may be absorbed by the microorganisms or may adhere to the microbial cells or to the feed particles and be lost during the process of centrifugation.

c) Aureomycin may be relatively unstable in the rumen. Thus, the structure may be altered, rendering detection impossible.

Assays also were run on the rumen liquor collected from two of the calves. As the calves did not eat all the concentrate mixture containing aureomycin supplement at one time, there was a more constant intake of aureomycin. Under these conditions the level of aureomycin in the rumen liquor was found to be 0.22 γ per milliliter.

It would seem from the in vitro cellulose digestion data that the cellulolytic organisms in the rumen may be inhibited by aureomycin. However, such a suppression was not suggested by the cellulose digester counts (Tables 4 and 5). Some resistance apparently develops when the organisms are subjected to the antibiotic over a long period of time, and the rapid inactivation of aureomycin in the rumen may account at least in part for the lack of continued response (either positive or negative) to aureomycin feeding in older animals. Furthermore, it is possible that the effect of aureomycin on cellulose digestion in vitro may not be typical of the response in vivo since growth, efficiency of feed utilization, and milk production of dairy cattle do not appear to be affected adversely by aureomycin feeding at moderate levels *(7, 13)*. Also, the results obtained with the purified cellulose may not be indicative of the response when natural feeds are the source of cellulose.

TABLE 4

Effect of aureomycin feeding on the microflora of the rumen[a]

Type of organism	Aureomycin-fed group	Control group
	(Plate counts/g of rumen ingesta, dry basis, $\times 10^3$)	
Lactate utilizers	788,578	32,310
Glucose fermenters	1,941	2,021
Cellulose digesters	5,674	4,588
Fungi	1,351	818
Coliforms	439	635
Proteolytic (gelatin liquefying)	71	69
Lactobacilli	17,750	17,241
Total aerobes	39,850	25,999
Total anaerobes	13,917	17,397
Clostridia	1,338	1,734

[a] Each group was composed of two cows and each value is an average of 8 to 14 determinations.

TABLE 5

Effect of the addition of aureomycin to the media on rumen microflora[a]

Cow group	Aureomycin in media	Lactate utilizers	Methyl cellulose utilizers	Glucose fermenters
		(Plate counts/g rumen ingesta, dry basis, $\times 10^4$)		
Control	None	1755	1773	832
	2.4 γ/ml	373	181	304
Aureomycin	None	1659	2292	1147
	2.4 γ/ml	610	486	607

[a] Each figure is an average of 12 values; three cows per group, two samples per cow, each sample run in duplicate.

There were no major differences between aureomycin and control animals with respect to the rumen populations of total aerobes, total anaerobes, clostridia, lactobacilli, proteolytic organisms, coliforms, fungi, cellulose digesters, glucose fermenters, thermophiles, and streptococci. Almost as much difference in rumen populations was found between samples taken from the same animal at different times and between samples taken simultaneously from animals on the same treatment as was shown between samples from aureomycin-fed and control animals. In the first series of observations the population of lactate utilizers appeared to be denser in the rumen of aureomycin-fed animals than in control animals (Table 4). In the second series, however, a similar difference was not observed (Table 5). The high counts on medium with lactate as the carbon source could indicate the presence of nutritionally fastidious organisms for which a highly supplemented glucose agar (such as used for total aerobes) is inadequate for growth.

The relative sensitivity to aureomycin of selected groups of rumen microorganisms taken from aureomycin-fed and control animals was tested by plating on appropriate media, with and without added aureomycin (2.4 γ per milliliter). The expected greater sensitivity to aureomycin of rumen microorganisms from control animals was noted (Table 5), although a marked depression occurred in both instances and the difference was not so marked as that observed for cellulose digestion in the artificial rumen.

For the lactate utilizer group from both aureomycin-fed and control groups there was a rapid decrease in numbers during the first few hours after collection of samples of rumen ingesta. Plate counts made after refrigeration of samples for 5 hours showed 90-99% decrease in population of these microorganisms from the population density found by plate counts which were set up within the first hour after collection. At the same time that this group of microorganisms was decreasing in numbers, mesophiles, notably *Proteus* and *Pseudomonas* species, were increasing rapidly in numbers in refrigerated rumen ingesta samples, as shown by plate counts.

From the rumen ingesta of cows that had received aureomycin continuously for 3 years, a variety of microorganisms was isolated that had much greater tolerance to aureomycin than did their counterparts isolated from rumen ingesta of control animals. *Monilia* and *Pseudomonas* species were isolated that were

able to grow in nutrient broth containing 1 mg. aureomycin hydrochloride per milliliter and that were shown to decompose this amount of aureomycin within 24 hours.

The effects of aureomycin on ruminating animals still are not entirely clear, particularly with reference to the changes that occur in the rumen microflora and microfauna when aureomycin is present in the ration. Methods that have proved successful for following population changes in less complex systems are inadequate for the study of rumen organisms. As indicated by Chamberlain *(2)*, the apparent populations of rumen flora as determined by plate counts vary with *(a)* the amount and quality of ration ingested, *(b)* the interval between feeding and time of sampling, *(c)* the mechanical treatment of the rumen ingesta prior to plating operations, and *(d)* the composition of the plating media. The apparent rapid disappearance of aureomycin from the rumen contents and the inability of some groups of rumen organisms to survive in rumen ingesta which have cooled below body temperature provide further complications.

SUMMARY

Rumen inocula from aureomycin-fed cows consistently digested, in the artificial rumen, a lower percentage of cellulose (73%) than those from control cows (85%). Rumen ingesta from 4-month-old calves receiving aureomycin digested 58% of the cellulose in the artificial rumen as compared to 78% digestion by inocula from control calves.

Aureomycin levels in the rumen liquid of the cows were 0.08 γ per milliliter 16 hours after, and 0.22 γ per milliliter 4 hours after, feeding 240 mg. of the antibiotic. The level in rumen liquid of the calves was 0.22 γ per milliliter approximately 2 hours after feeding 40 mg. of aureomycin (one-half of the daily allowance).

The addition of 2.4, 1.6, 0.8, and 0.4 γ, respectively, of aureomycin per milliliter of fermentation mixture in the artificial rumen severely inhibited cellulose digestion by the microorganisms from the control cows. The same additions had comparatively little effect on the digestion of cellulose by inocula from the aureomycin-fed cows.

Plate counts also indicated that rumen microflora are affected by the administration of aureomycin, but most of the inhibitory effect is lost after a period of adjustment by the microorganisms. The emergence of strains of *Pseudomonas* and *Monilia* that have the ability to inactivate aureomycin was demonstrated, which may explain the lack of positive differences between aureomycin-fed and control cows in weight gains, milk production, and efficiency of feed utilization.

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FERTILIZATION AND EMBRYONIC MORTALITY RATES OF BULLS WITH HISTORIES OF EITHER LOW OR HIGH FERTILITY IN ARTIFICIAL BREEDING

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Because of the complexity of the reproductive process, instances of its failure undoubtedly represent a multiplicity of causes. Consequently, an attack on the problem of infertility requires that the problem be broken down into segments subject to objective study. Until recently there has been little evidence in cattle to indicate what proportion of the infertile matings are caused by nonfertilization and what proportion are caused by early death of the conceptus.

The literature pertaining to the cow indicates that although she produces a high proportion of fertilizable eggs, she is a major contributor to early embryonic mortality. The work of Kidder *et al. (3)* indicates that the infertile matings by high fertility bulls are primarily embryonic failures, whereas those by low fertility bulls are mostly failures of fertilization. From studies of the differences between 1-month and 5-month nonreturns in artificial insemination, Salisbury *et al. (10)* presented evidence indicating that the bull contributes to early embryonic mortality. Finally, the studies by Foote and Bratton *(2)* of delayed returns in artificial insemination before and after the additions of antibiotics to extended semen suggest that the semen per se is the origin of some of the delayed returns. This evidence, along with the work of McEntee *et al. (5)* and Plastridge *et al. (9)*, showing that a major characteristic of vibriosis is early embryonic mortality, strongly suggests that the improved fertility that has accompanied the addition of antibiotics in unfrozen semen has been due, in part, to the control of this infection.

The investigation reported in this paper was designed to obtain direct quantitative information regarding the extent to which the bull may be responsible for the nonfertilization of ova and for the early death of the conceptus in known vibriosis-free test heifers inseminated with extended semen containing penicillin and streptomycin.

EXPERIMENTAL PROCEDURES

The general plan of the experiment was to breed heifers artificially with semen from bulls having histories of either high or low 60- to 90-day nonreturns to first services in artificial breeding. Half of the heifers bred to each bull were slaughtered 3 days post-estrus to determine the percentage of ova fertilized, and the others were slaughtered 33 days post-estrus to determine the percentage of normal embryos present. For a particular group of bulls the difference between the average percentage of ova fertilized and the average percentage of normal embryos was considered to be embryonic mortality.

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The bulls studied were in the active stud of the New York Artificial Breeders' Cooperative, Inc. There were eight Holsteins in the "high" fertility group and one Holstein, four Guernseys, and one Ayrshire in the "low" fertility group. The average difference in the 60- to 90-day nonreturns to first services between the two groups at the outset of the experiment was 16 percentage units. The semen from these bulls used to breed the experimental heifers was taken from that which was processed for field shipment by the Cooperative. The extender consisted of equal parts of fresh egg yolk and 2.9% sodium citrate buffer containing 0.6% of sulfanilamide *(1)*. Five hundred units each of penicillin and streptomycin were added per milliliter of extender. The average number of motile spermatozoa per milliliter of extended semen was 13.3×10^6 for the low fertility bulls and 10.7×10^6 for the high fertility bulls. One ml. of this semen was used to inseminate each experimental heifer. The average age of the semen at the time of use was 18 hours for the low fertility bulls and 13 hours for the high fertility bulls. The age of the semen used for breeding cows in the field artificially was estimated to average 33 hours for both groups of bulls.

Vibrio fetus organisms were isolated from the semen of three of the low fertility and five of the high fertility bulls. But in view of the work by McEntee *et al. (5, 6)* showing that the addition of antibiotics to naturally infected semen prevented virgin heifers from becoming infected, and the in vitro studies by Orthey and Gilman *(7, 8)* showing that antibiotics added to contaminated semen inactivated *V. fetus* organisms, it was believed that the use of the extended, antibiotic-treated semen of the infected bulls would not infect the experimental heifers and therefore would not contribute to embryonic mortality.

The experimental heifers were virgins and free from genital abnormalities. All were blood tested for brucellosis and vibriosis soon after arrival at the experimental barn. During the first or second heat period thereafter, a vaginal mucus sample was taken for *V. fetus* culture. At the time of slaughter another vaginal mucus sample was taken for a vibrio agglutination test, and the contents of one uterine horn were cultured for *V. fetus* organisms. All heifers were negative to these tests.

At the time of breeding, two of the heifers were about 30 months of age and the remainder were 14 to 24 months of age. Daily observations for signs of estrus were made at approximately 7:00 A.M., 9:00 A.M., 11:00 A.M., 2:00 P.M., and between 7:00 and 9:00 P.M. All heifers were allowed to have at least one control estrus cycle before being inseminated. Inseminations were made by the senior author as close to the middle of the estrous period as possible. None were made earlier than 6 hours after the beginning of estrus and none after the end of estrus. The semen was deposited either in the body of the uterus or in the anterior end of the cervix.

The heifers that were scheduled for slaughter 33 days post-estrus and had not returned to estrus by the 21st day post-estrus were palpated per rectum between the 21st and 23rd day to detect possible ovulations without associated estrus. Heifers that had a large corpus luteum and no large follicles at this time were considered to be pregnant and were not repalpated before slaughter-

ing. In cases of doubt as to the state of the ovary at this time another palpation was made 2 to 4 days later.

The eggs from the heifers slaughtered 3 days post-estrus were flushed from the oviduct and examined at a magnification of 100× to determine the state of cleavage, and those with two or more blastomeres of approximately equal size were classified as fertilized. However, it was recognized that nondetectable aberrations may have occurred up to this stage of cleavage, which in turn might affect the subsequent viability of the embryo.

To recover the embryos at 33 days post-estrus the heifers were stunned and bled, and the uteri were removed immediately and kept at body temperature until the embryos were removed. The embryos were removed within 10 minutes. The first three embryos recovered were judged to be alive and normal on the basis of gross morphological examination, but for the remainder the heartbeat was used as the principal evidence of life at the time of removal.

RESULTS AND DISCUSSION

The fertilization rates and the embryonic survival and mortality rates for the two groups of bulls are shown in Table 1. Of particular interest here is the high fertilization rate, 96.6% for the high fertility group as compared to 76.9% for the low fertility group. The difference of 19.7 percentage units had a probability of 0.07 when tested by Chi-square. The embryonic mortality rate for the high bulls (10.5%) was about one-half that for the low bulls (19.6%). The difference of 9.1 percentage units between the two groups of bulls had a P value greater than 0.05. It should be noted that the relatively low embryonic mortality rate in these normal heifers bred to high fertility bulls is in contrast to the high embryonic mortality rates (36 to 43%) reported by Tanabe and Almquist *(11)* and Tanabe and Casida *(12)* for repeat breeding cows and heifers bred to high fertility bulls.

All of the six unfertilized eggs in the 3-day low fertility bull group were from matings to bulls 125 and 609. If one assumes that for a particular bull the evidence for nonfertilization in the 3-day group is valid evidence for nonfertilization in the 33-day group, the five missing embryos for these two bulls probably were not embryonic deaths, since they could be accounted for by the rate of nonfertilization. The four remaining bulls in the low group had one or more missing embryos which were considered to be embryonic mortalities, since all of these bulls' matings to 3-day slaughter heifers resulted in fertilized eggs.

There were one abnormal embryo and three missing embryos in the high bull group and one unfertilized egg, leaving three presumed early embryonic mortalities.

The single unfertilized egg recovered from the group of high fertility bulls and one of the six from the group of low fertility bulls consisted of only an empty zona pellucida. All others appeared to be normal. Kidder *et al. (3)* have described four such abnormal eggs recovered from 74 heifers slaughtered 3 days post-estrus. However, they excluded this abnormal type in calculating their estimates of embryonic mortality.

TABLE 1

Fertilization, embryonic survival, and embryonic mortality rates of bulls with histories of either high or low fertility in artificial breeding

Bull No.	Heifers slaughtered 3 days post-estrus		Heifers slaughtered 33 days post-estrus		No. of probable embryonic mortalities
	No. of fertilized eggs	No. of unfertilized eggs	No. of normal embryos	No. of missing embryos	
"High" fertility bulls: (29 heifers per group)					
811	1	0	0	1	1
611	4	0	2	2	2
502	4	0	4	0	0
410	5	0	4	1	1
308	3	0	3	0	0
701	4	0	4	0	0
802	6	1	7	0	−1[a]
706	1	0	1	0	0
Total	28	1	25	4	3
Per cent	96.6	3.4	86.1	13.9	10.5
"Low" fertility bulls: (26 heifers per group)					
125	0	2	0	2	0
609	7	4	7	3	−1[a]
833	6	0	5	1	1
726	2	0	1	1	1
821	1	0	0	1	1
761	4	0	2	3	3
Total	20	6	15	11	5
Per cent	76.9	23.1	57.7	42.3	19.6
Differences between groups:					
High-low	19.7 ($P = .07$)		28.4 ($P < .05$)		
Low-high					9.1 ($P > .05$)

[a] The −1 results from the subtraction of column 4 from column 2.

The percentages of 60- to 90-day nonreturns to first services for each bull before and during the experimental breeding periods are shown in Table 2. With the exception of bulls 726 and 833, the average nonreturn rates for each bull during the two periods were remarkably similar. Bull 726 was mated to only four heifers because his use was discontinued as soon as it became apparent that his nonreturn rate during the experimental breeding period was similar to that of the bulls in the high group. The nonreturn rate for bull 833 became lower as the experiment progressed.

Histological examinations of post-mortem material from the heifers revealed no significant pathology, there being only 21 cases of mild endometritis consisting of scattered lymphocytes and 25 cases with occasional cystic uterine glands. These types of abnormalities were divided about equally between the groups bred to the high and to the low fertility bulls and between the 3- and 33-day slaughter groups. It therefore seems unlikely that they were the cause of the difference in reproductive failures between the two groups of bulls.

The embryo heartbeat rate was extremely variable, ranging from 20 to 116 per minute, and may be partly accounted for by the fact that small temperature

TABLE 2

Fertility levels of bulls before and during experimental breeding period

Bull No.	5-month period before experimental breedings		From experimental breedings	
	1st serv.	60- to 90-day N.R.	1st serv.	60- to 90-day N.R.
"High" fertility bulls:		*(%)*		*(%)*
811	6,636	74	748	74
611	1,543	75	1,447	74
502	8,059	75	2,999	73
410	6,029	75	3,015	76
308	2,046	75	2,411	73
701	2,108	76	2,747	76
802	9,257	75	4,655	73
706	1,180	75	58	79
Total	36,858		18,080	
Average		75		74
"Low" fertility bulls:				
125	968	41	53	44
609	646	58	475	57
726	1,054	64	631	76
761	276	60	242	64
821	2,503	64	55	65
833	2,129	63	368	42
Total	7,576		1,824	
Average		58		58

changes at the time of observation caused large fluctuations in the heartbeat rate. For example, raising the bath temperature from 30° to 35° C. increased the rate from 60 to 90 beats per minute. On the other hand, six embryos held at a constant temperature of 35° C. varied only from 84 to 95 beats per minute.

The state of cleavage of the fertilized eggs from the heifers bred to the low fertility bulls ranged from two to 16 cells with a mean of 6.8 and a median of 8.0. Those from the heifers bred to the high fertility bulls ranged from four to 16 cells with a mean of 8.3 and a median of 8.0.

Crown-rump measurements of the embryos were made first in their amnions and then after fixation in 10% formalin. The mean post-fixation lengths of the embryos was 11.0 mm. $\pm$ 1.51 and 10.6 mm. $\pm$ 1.67 for the high and low fertility groups, respectively. The mean post-fixation weights of the embryos were 186.2 mg. $\pm$ 70.6 and 162.3 mg. $\pm$ 79.7 for the high and low fertility groups, respectively. The differences in lengths and weights for the two groups of bulls had a P value greater than 0.05. These large variations in the length and weight of 33-day embryos cast considerable doubt on the validity of determining the age of slaughterhouse embryos from their length and/or weight.

In view of the high fertilization rates of the high fertility bulls used in this experiment and the results reported by Laing *(4)* and Kidder *et al. (3)* it seems reasonable to conclude that, in general, bulls with 60- to 90-day nonreturn rates of 70% or higher when bred to normal females at the proper time during estrus have fertilization rates that are essentially 100%, the few instances of nonfertilization being a reflection of the cows producing nonfertilizable eggs. Conse-

quently, almost all repeat breedings associated with high fertility bulls would reflect early death of the conceptus. In contrast, repeat breedings associated with bulls with low fertility present a dual problem; low fertilization rates accompanied by little, if any, embryonic mortality, or high fertilization rates, accompanied by high early embryonic mortality rates. These two kinds of low fertility bulls when considered together may introduce so much variation within experimental groups that statistically significant differences between groups cannot reasonably be expected when relatively small numbers of test heifers are used. In fact, if the variation encountered in this study is generally true for this criterion of infertility, it would require approximately 90 heifers per experimental group to obtain statistical significance at the 0.05 level of probability. This large requirement for test females raises a serious question as to how a substantial amount of direct evidence can be obtained on these possible points of failure in the reproductive process to establish them as highly probable fact or fiction without the expenditure of considerably larger amounts of time and money than have been thought necessary in the past.

Since the evidence for the embryonic mortalities in this experiment consisted almost entirely of missing embryos at 33 days post-estrus, it appears that the embryos were dying at an age too early to allow recovery of disintegrating remnants. It follows, therefore, that future research should be centered around an earlier period in the gestation than 33 days post-estrus.

SUMMARY AND CONCLUSIONS

On the basis of 3-day post-estrus slaughter data the fertilization rate was 76.9% for 26 heifers bred to bulls with histories of "low" fertility in artificial breeding and 96.6% for 29 heifers bred to bulls with histories of "high" fertility in artificial breeding. The percentage of normal embryos from the same number of heifers bred to the low fertility bulls and slaughtered 33 days post-estrus was 57.7 and the percentage of normal embryos from the same number of heifers bred to the high fertility bulls and slaughtered 33 days post-estrus was 86.1. The percentage of heifers not pregnant, presumably because of embryonic mortality, was 19.6 for the low fertility bulls and 10.5 for the high fertility bulls. The difference of 9.1 percentage units between the two groups of bulls is not statistically significant. It would appear from the data that with high fertility bulls, instances of repeat breedings are nearly always embryonic mortalities, whereas with bulls of low fertility both nonfertilization and early embryonic mortality occur.

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ANTIBIOTICS IN DAIRY CATTLE NUTRITION. VI. THE EFFECTS OF AUREOMYCIN[1] FEEDING ON THE APPARENT DIGESTIBILITY OF CERTAIN CALF RATIONS[2]

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The increased growth rate and improved feed efficiency that result from feeding aureomycin to dairy calves *(2, 18)* suggested an investigation of the effect of aureomycin supplement on the digestibility of calf rations. Such a study might help in determining the mode of action of the antibiotic.

Since the present study was started, Murley *et al. (14)* have reported that the addition of aureomycin to a dairy calf ration resulted in a slightly, but not significantly, greater efficiency of "utilization" of carbohydrates, nitrogen, ash, and ether extract. Hibbs *et al. (10)* found that the addition of aureomycin to a hay and grain ration did not affect the digestion of dry matter, cellulose, and protein by 12-week-old calves.

Studies on the effects of antibiotics on the digestibility of nutrients by non-ruminants have produced a number of conflicting reports *(3-8, 11, 16, 19, 20)*.

EXPERIMENTAL PROCEDURE

Twelve male calves (six Holsteins, four Jerseys, and two Guernseys) were paired by breed and age. Each pair was assigned at random to one of three ration groups. The calves in Group 1 received milk only, calves in Group 2 received milk and oats, and those in Group 3 received milk and alfalfa hay. From birth, crystalline aureomycin HCl was given daily by capsule at the rate of 25 mg. per 100 lb. body weight to one member selected at random from each pair; the other served as the control.

The calves were removed from their dams at 3 days of age and housed in individual pens in the College calf barn. All calves then were bucket-fed whole mixed herd milk in two equal feedings at the daily rate of 10 lb. per 100 lb. of body weight. The calves were weighed weekly, at which time adjustments were made in quantities of milk fed. Beginning at 19 days of age, calves in Group 2 were fed oats ad libitum and those in Group 3 were fed hay ad libitum. Four days prior to the start of the digestion trials, the calves were placed in metabolism cages and fitted with feces collection bags to accustom them to the surroundings and equipment used during digestion trials. A battery of metabolism cages was used. The individual cages, $4 \times 4 \times 4$ feet in size, were mounted on a platform 18 in. above the floor. The bottoms of the cages were made of

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5/8-in. mesh, 16 gauge extruded metal. The calves were muzzled during the digestion trials, except during feeding periods. Feces were collected in plastic liners inserted in small canvas bags constructed to fit around the tail and over the rump of the calf. The bags were held in place by adjustable harness *(15)*. The plastic liners were changed at least twice daily and the feces were refrigerated until the end of each collection period, when a composite sample was prepared for analysis.

Milk, grain, hay, and feces samples were analyzed for moisture, crude protein, ether extract, ash, and crude fiber according to A.O.A.C. methods for feeds *(1)*, except that milk and feces were mixed with anhydrous sodium sulfate by grinding to absorb moisture prior to extraction of fat.

Digestion Trial I was started when calves were 53 days of age. This trial consisted of a 10-day preliminary period, followed by a 7-day collection period. At the end of the collection period the calves were returned to the calf barn. A second digestion trial was started, with the same calves fed the same feeds, when the calves were 81 days of age. This trial also consisted of a 10-day preliminary period and a 7-day collection period. During the digestion trials, while the calves were in the metabolism cages, they were fed herd milk standardized to 3% fat content. This milk was fed at the same rate as that just previous to the trial, except that no adjustment of quantity fed was made during the trial. The calves in Groups 2 and 3 were fed grain and hay, respectively, at the rate of 200 g. per 100 lb. body weight daily during digestion Trial I and 400 g. per 100 lb. body weight daily during digestion Trial II. The grain was medium ground oats of good quality and the hay was coarse ground alfalfa of good quality. All calves received daily, by capsule, 400 units of vitamin D.

RESULTS AND DISCUSSION

The data on digestibility of the rations are presented in Table 1. Administering aureomycin improved slightly the digestibility of the various nutrients when the calves were fed milk only, but, in general, differences were so small that they could be of only minor importance.

The digestion of the nutrients of the milk and oats ration fed calves during Trial I (9 weeks of age) was changed little when aureomycin was given, except that data on crude fiber cannot be interpreted because of an unexplained negative digestion coefficient obtained with calf 025A. The digestion of most nutrients of the milk and oats ration during Trial II (13 weeks of age) was only slightly lower when calves received aureomycin, but digestion of ash was about 10% lower and of crude fiber, 60% lower.

When aureomycin was given to calves fed the milk and hay ration during Trial I, digestion of the crude fiber and other nutrients was similar to digestion of crude fiber and other nutrients by calves on the milk and oats ration during Trial II, except that one aureomycin-supplemented calf digested N.F.E. better than either of the controls. During Trial II digestibility of the nutrients in the milk and hay ration was essentially the same by calves receiving and those not receiving aureomycin.

TABLE 1

The effect of aureomycin on the digestibility of milk, milk and oats, and milk and alfalfa hay by dairy calves

Ration	Pair	Calf No.	Supplement	Apparent digestion coefficients											
				Digestion trial I[a]						Digestion trial II[a]					
				Dry matter	Crude protein	Ether extract	N.F.E.	Ash	Crude fiber	Dry matter	Crude protein	Ether extract	N.F.E.	Ash	Crude fiber
Milk	I	0151B	Aureo.[b]	98.5	98.1	99.2	99.3	92.9		98.7	95.5	98.3	99.0	88.5	
		0152B	None	98.2	97.1	98.9	99.5	92.8		95.8	94.6	97.4	98.1	81.6	
	II	0341B	Aureo.	97.5	95.9	99.6	99.5	92.0		96.4	94.9	97.7	98.0	87.7	
		027A	None	94.0	89.7	97.3	96.6	77.0		94.4	93.0	95.2	98.1	86.4	
		Av.	Aureo.	98.0	97.0	99.4	99.4	92.5		97.5	95.2	98.0	98.5	88.1	
		Av.	None	96.1	93.4	98.1	98.1	84.9		95.1	93.8	96.8	98.1	83.5	
	Av. daily intake *(g.)*			3750	1112	1034	1358	245		5200	1488	1521	1853	338	
Milk and oats	I	025A	Aureo.	86.7	92.0	98.3	86.2	75.6	−28.1	86.4	91.2	97.2	88.4	73.6	19.8
		026A	None	90.2	91.9	98.8	86.8	77.3	18.3	90.1	92.9	97.7	92.6	83.9	53.2
	II	0227B	Aureo.	90.5	93.5	98.9	86.5	81.3	27.3	86.7	90.8	96.9	88.5	76.3	16.2
		0228B	None	90.1	93.7	98.2	90.8	79.7	22.6	90.4	92.8	97.2	91.9	82.1	38.7
		Av.	Aureo.	88.6	92.8	98.6	86.4	78.5	−0.9	86.6	91.0	97.1	88.4	74.6	18.0
		Av.	None	90.2	92.8	98.6	88.8	78.5	20.5	90.3	92.9	97.5	92.3	83.0	46.0
	Av. daily intake *(g.)*			4457	1071	905	2083	261	129	7432	1639	1139	3958	387	313
Milk and alfalfa hay	I	0153B	Aureo.	79.0	82.8	92.7	83.1	65.7	20.0	82.1	85.0	94.7	85.9	73.4	46.5
		0154B	None	81.9	83.3	93.8	85.0	67.9	42.0	82.6	85.1	93.7	86.2	74.1	47.0
	II	0157B	Aureo.	71.1	77.4	89.4	96.2	51.2	10.3	83.5	85.9	94.6	86.1	69.4	50.0
		0156B	None	81.1	83.5	93.0	86.1	68.0	41.2	82.1	85.4	93.6	87.5	70.3	41.6
		Av.	Aureo.	75.1	80.1	91.1	89.6	58.4	15.2	82.8	85.5	94.7	86.0	71.4	48.3
		Av.	None	81.5	83.4	93.4	85.5	68.0	41.6	82.4	85.3	93.7	86.9	72.2	44.3
	Av. daily intake *(g.)*			6843	1771	1199	2734	535	602	10153	2482	1630	4180	761	1099

[a] At the start of the first and second digestion trials the calves were 9 and 13 weeks old, respectively.

[b] Administered at the rate of 25 mg. per 100 lb. body weight daily.

The increased digestibility of crude fiber when the larger quantity of hay was fed in the milk and hay ration to older calves given aureomycin in Trial II seems inconsistent with the depressing effect of aureomycin on the digestibility of crude fiber when either the smaller quantity of hay (Trial I) or even the same weight of oats as hay (Trial II) was fed. Studies cited below, however, offer possible explanations for these findings.

Several workers *(9, 12, 13, 17)* have reported that aureomycin decreased the digestibility of pure cellulose by rumen bacteria in vitro; but Radisson *(17)* found that certain feedstuffs counteracted the depression of cellulose digestion in the presence of aureomycin. He found that alfalfa hay was more effective than a grain mixture. Further, Hanold *(9)* observed in similar studies that grains differed, oats being less effective than corn, milo, or barley. Thus, the results of the present study might be interpreted as follows: Aureomycin possibly affects digestion of nutrients through an effect on cellulose-digesting organisms. When feeds containing cellulose were left out of the ration or were present in small quantities (milk alone, Trials I and II; milk and oats, Trial I), the administration of aureomycin did not materially decrease digestion of nutrients. In fact, when calves were fed milk alone, digestion was improved slightly. When larger quantities of oats or hay were included in the ration (milk and oats, Trial II; and milk and hay, Trial I), aureomycin may have affected the cellulose digesting organisms, resulting in less complete digestion of fiber than when aureomycin was absent. Also, digestion of certain other nutrients indirectly may have been decreased somewhat, because of the "encrusting" effect of fiber. When, however, still larger quantities of alfalfa were added to the ration of older calves receiving aureomycin (milk and hay, Trial II), some factor or factors of alfalfa counteracted the depressing effect of aureomycin in a manner similar to that found in the in vitro experiments.

Another factor which might have contributed to these general results is a greater development of the rumen in the hay-fed calves, particularly at the older age. The calves fed oats, and those fed hay, were started on equal quantities of these respective feeds when 19 days of age. They received the same weights of oats or hay during Trial I (9 weeks of age) and the same respective increased quantities during Trial II (13 weeks of age). However, during the first trial the calves fed alfalfa received 4.5 times as much crude fiber as those fed oats (Table 1), and during Trial II, the calves fed alfalfa received 3.5 times as much crude fiber as those fed oats. Also, the calves fed alfalfa in Trial I received twice as much fiber as the calves fed oats in Trial II. Since both oats and hay were fed ad libitum previous to each trial, even greater differences in crude fiber intake may have prevailed during the periods preliminary to the digestion trials than during the trials. The quantity of fiber or roughage in the diet is believed to influence rumen development *(10)*, and, therefore, greater rumen development when alfalfa is fed might be expected. Possibly the greater the rumen development, the less the effect of aureomycin. Results *(18)* on older calves support this deduction.

Results of these trials, when milk alone was fed, are in harmony with those of Murley *et al. (14)*, who found a slight but nonsignificant increase in digestibility of nutrients when aureomycin was included in the diet of calves fed reconstituted skimmilk at 2, 5, and 8 weeks of age. Also, the results reported here are consistent with those of Hibbs *et al. (10)*, who fed 12-week-old calves no milk but grain and mixed alfalfa-clover-timothy hay and reported that the addition of aureomycin to the ration had no effect on digestion of dry matter, cellulose, or protein.

The growth and feed efficiency of the calves on these trials continuously receiving aureomycin from birth to 12 weeks of age were greater than those of control calves receiving no aureomycin (Table 2), which is in harmony with

TABLE 2

Effect of aureomycin feeding on growth and efficiency of gain of calves from birth to 12 weeks of age

Ration	Supplement	No. of calves	12th wk. wt. in % of birth wt.	lb. TDN/lb gain
Milk only	Aureomycin	2	175	2.3
	None	2	167	2.8
Milk and oats	Aureomycin	2	199	1.9
	None	2	177	2.3
Milk and alfalfa	Aureomycin	2	186	2.4
	None	2	173	2.7

reports by others *(2, 10, 18)*. It would seem from results reported herein that the general mode of action of aureomycin in promoting growth and better feed efficiency is not the result of improvement in digestion of rations when this antibiotic is given.

SUMMARY AND CONCLUSIONS

Twelve male dairy calves were used in a study to determine the effect of administering aureomycin on the digestibility of rations of milk only, milk and oats, and milk and alfalfa hay. Digestion trials were conducted when the calves were 9 weeks old and again when they were 13 weeks old.

When calves were fed only milk, the administration of aureomycin improved slightly the digestibility of the various nutrients, but differences were so small that they could be of only minor importance.

When rations of milk and oats or milk and alfalfa hay were fed, there were no marked or consistent effects of aureomycin on digestibility of nutrients, except crude fiber. Aureomycin appeared to have reduced digestibility of crude fiber by 13-week-old calves fed milk and oats and by 9-week-old calves fed milk and alfalfa hay but did not reduce digestibility of crude fiber by 13-week-old calves fed milk and alfalfa hay.

The greater digestion of crude fiber by calves receiving aureomycin and fed the larger quantity of alfalfa hay is in harmony with findings in in vitro experiments, which indicated a superiority of alfalfa hay compared with some other

feeds in counteracting the depressing effect of aureomycin on digestion of nutrients. Also, differences in fiber intake may have caused differences in rumen development, which in turn may have influenced digestion of fiber. The findings reported herein are additional evidence that the beneficial effects of aureomycin on growth and feed efficiency are not due to improvements in digestibility of feeds.

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METABOLISM OF BULL SEMEN. II. FRUCTOLYSIS RELATIONSHIPS WITH SPERM CONCENTRATION AND FERTILITY[1]

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The identification of fructose as the principal sugar in semen *(14, 20)* was accompanied by studies on several species, including the bull, which showed the accessory sex glands as sole contributors *(11, 14, 25)*. Citric acid *(24)* and fructose *(11, 13, 14, 24)* content of semen has been suggested as an indicator of male sex hormone activity, though this has been questioned from studies of some subfertile bulls. In one such study *(3)* a failure of secretory structures to respond to the hormone was suggested.

Levels of fructose initially present in bull semen vary from 2 to 12 mg. per milliliter of semen *(1-6, 8, 10, 16-19, 22, 24, 25)*.

Rate of fructose utilization *(18, 19)* by normal semen was first reported to be 1.4 to 2.0 mg. per hour per 10^9 sperm at 37° C. and has been since substantially confirmed *(2, 10, 12, 16, 17, 20)*.

The relationship of levels of fructose initially present in semen and fructose utilization during periods of incubation from 1 to 3 hours at 37° C. with fertilizing capacity is not clear, though most reports *(1, 2, 3, 8, 10, 11, 16, 17, 19, 24, 27, 29)* agree that such comparisons have diagnostic value.

Fructose utilization during comparable periods of incubation increases as sperm concentration increases *(2, 16-19, 24, 29)*, while initial levels of fructose decline because of the greater space occupied by the sperm cells which contain little or no fructose *(24)*.

Gassner *et al. (15)* and Hopwood *et al. (17)* determined fructose utilized by 135 semen samples at incubation periods varying from 20 minutes to 3 hours at 37° C. When concentration of fructose was plotted against time, a smooth decay curve resulted, whereas the log of fructose concentration yielded a linear function. These findings indicated that fructose utilization followed the curve of a first order reaction.

The high relationship between rate of utilization of fructose and sperm concentration plus the association of above-average sperm concentration and below-average levels of initial fructose makes comparison of fructolysis studies difficult. Cupps *et al. (6)* have observed exhaustion of fructose in some samples incubated at 37° C. for 2 hours.

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The amount of seminal fructose required for sustained fructolysis during incubation periods of 1 hour or more at 37° C. exceeds the quantities utilized during storage for 24 hours at 10° C. *(4)*. Thus, under the conditions usually employed for the fructolysis test, semen samples of low sperm concentration tend to be overevaluated compared with samples of high initial sperm concentration.

In order to clarify some of the factors interfering with the evaluation of fructose utilization studies, the data of Gassner *et al. (10, 11, 13, 15, 17)* were combined with similar data from the State College of Washington. The purpose of this study was to establish relationships between *(a)* levels of fructose before incubation, *(b)* fructose utilization during incubation, *(c)* sperm concentration, and *(d)* fertilizing capacity of semen.

MATERIALS AND METHODS

Colorado. Three separate experimental periods (1949-52) involving 390 semen samples from 29 bulls (44 bull-periods) of six breeds have been previously and separately reported *(13, 15, 17)*. Too few services per sample precluded analysis on other than a between-bull basis. Nonreturns for first and second services (60 to 90-day) were calculated by using total services and nonreturns for each bull during the experimental period. Semen was collected on alternate days. Dilution was with egg yolk–citrate containing antibiotics. Rates of dilution did not exceed 1 : 40. Approximately 75% of the services were with semen collected the previous day.

Semen for determination of initial fructose and fructose utilization was handled in the following manner. When the semen was received in the laboratory, duplicate samples of 0.8 ml. were diluted with 0.4 ml. of 0.125 *M* Na_2HPO_4 buffer (pH adjusted to 7.4 with 0.1 *N* HCl). After gentle mixing, the samples were incubated at 37° C. for 3 hours *(17)*. Fructose was determined by the method of Hopwood *et al. (17)*.

Washington. From June, 1952, through January, 1953, 513 semen samples were collected from 36 bulls (four breeds) in routine artificial service at Northwest Co-op Breeders, Burlington. Four hundred of the 513 samples were used for more than 20 first and second services and for the within-bull analyses involving fructose, fructolysis, and nonreturn. Semen was collected daily, except Sunday, and diluted with heated homogenized milk at a rate giving in excess of 10^7 sperm per milliliter of diluted semen. Seventy per cent of the services occurred the day after collection. Nonreturns were determined for all first and second services (60 to 90-day).

Immediately after collection of semen, 0.5 ml. was added to 2.0 ml. of 2.9% Na-citrate buffer at 37° C. One ml. of this mixture was forcibly discharged into 19 ml. of 10% trichloroacetic acid in tubes maintained in an ice water bath for determination of zero time (initial) fructose; this process was repeated at the termination of 60-minute incubation at 37° C. of the 1:4 semen to Na-citrate mixture to give a zero-hour and 1-hour determination of fructose.

Fructose was determined by the method of Roe *(26)*, modified by taking 2 ml. of the trichloroacetic acid filtrate, representing 0.02 ml. of semen, and adding 6 ml. of a solution containing 80% 10 *N* HCl and 0.034 g. of resorcinol per 100 ml. Colorimetric determinations were made with an Evelyn colorimeter with a wave length of 520 mμ.

Colorado and Washington. An incubation time of 1 hour was found to be too long to accurately appraise the glycolytic capacity of some semen samples because of combinations of low initial fructose, high sperm concentration, and unexpected utilization rates. Five samples showed less than 0.1 mg. of fructose remaining after 1 hour. These were discarded from the summaries to follow.

By using the first order reaction formula, $K = \frac{2.3}{t} \log \frac{\text{initial fructose}}{\text{fructose at 1 hour}}$ and $t\,½ = \frac{0.693}{K}$ [the time required to use one-half ($t\,½$) of the initial fructose present in each semen sample], the half-life of the 898 remaining samples ranged from 12 minutes to 11½ hours.

In order to place all samples on a comparable basis, maximum utilization rate was determined by calculating the amount of fructose remaining after 10 minutes of incubation where $t\,⅙ = \log \text{initial fructose} - \left(\frac{K}{2.3} \cdot t\,⅙\right)$ and fructose utilized during the first 10 minutes of incubation = initial fructose − fructose remaining at 10 minutes of incubation. Utilization rate during the first 10 minutes of incubation $= \frac{\text{utilization rate}}{10^9 \text{ sperm/ml}}$.

This yielded a fructolysis index which estimated the fructolytic capacity under conditions where fructose availability and variable concentration of sperm were not limiting factors.

Since all or nearly all fructose is present in the seminal plasma *(24)*, initial fructose was changed from a whole semen basis to a value estimated for semen less sperm cell volume *(9)*.

Statistical analyses were as outlined by Snedecor *(30)*.

RESULTS AND DISCUSSION

Similarity of the data. Comparisons for breeds and states (Table 1) reveal marked differences for the several criteria shown. Breed comparisons for levels of initial fructose show Guernseys highest for Washington and lowest for Colorado, whereas Colorado Jerseys exceeded Washington Jerseys. Other breed differences tended to follow a consistent pattern when the variability of sperm concentration is considered. The results indicate the difficulty of establishing real breed differences for semen characteristics.

Averages for states show that sperm concentration was 55% and utilization of fructose during 60 minutes incubation 54% as high for Colorado as Washington, yet the utilization rate for 10^9 sperm per hour was 11% higher for Colorado. Fructose utilization for 10^9 sperm for 10 minutes was not significantly different.

TABLE 1

Averages by breeds within states for the semen characteristics under study

Source		Semen samples	Sperm conc.	Semen fructose						Seminal plasma fructose *(9)*	
				Initial	1 hour	Utilization					
						1 hour		10 minutes[a]		Initial	1 hour
		(No.)	*(10^9/ml)*	*(mg/ml)*	*(mg/ml)*	*(mg/ml)*	*(mg/10^9 sperm)*	*(mg/ml)*	*(mg/10^9 sperm)*	*(mg/ml)*	*(mg/ml)*
Colorado		389	0.93	5.03	2.97	2.06	2.28	0.45	0.49	5.44	3.20
Washington		509	1.67	6.11	2.95	3.16	2.05	0.83	0.50	7.01	3.31
Total		898	1.34	5.65	2.96	2.69	2.15	0.67	0.50	6.34	3.26
Colorado % of Washington		av.	55.4	82.00	99.00	65.00	111.00	54.00	98.00	77.00	97.00
Colorado	Holstein	125	0.99	5.61	3.44	2.17	2.23	0.46	0.47	6.10	3.72
Washington	Holstein	161	1.70	6.12	2.81	3.31	2.13	0.86	0.52	7.05	3.18
Total	Holstein	286	1.39	5.90	3.09	2.81	2.17	0.68	0.50	6.63	3.42
Colorado	Guernsey	133	0.95	4.50	2.41	2.09	2.20	0.50	0.50	4.87	2.60
Washington	Guernsey	156	1.39	7.40	4.40	3.00	2.25	0.65	0.48	8.31	4.92
Total	Guernsey	289	1.19	6.07	3.49	2.58	2.23	0.58	0.49	6.73	3.85
Colorado	Jersey	24	1.24	5.25	3.10	2.15	1.77	0.46	0.37	5.82	3.42
Washington	Jersey	144	1.99	5.01	1.42	3.59	1.90	1.09	0.56	5.94	1.65
Total	Jersey	168	1.88	5.05	1.67	3.38	1.88	1.00	0.53	5.92	1.91
Washington	Hereford	48	1.46	5.37	3.17	2.20	1.63	0.54	0.36	6.06	3.52
Colorado	Brown Swiss	8	0.90	5.10	2.99	2.11	2.43	0.44	0.51	5.49	3.22
Colorado	Milking Shorthorn	99	0.74	4.96	3.10	1.86	2.58	0.38	0.53	5.28	3.30

[a] Estimated from 1-hour utilization based on first order reaction formula.

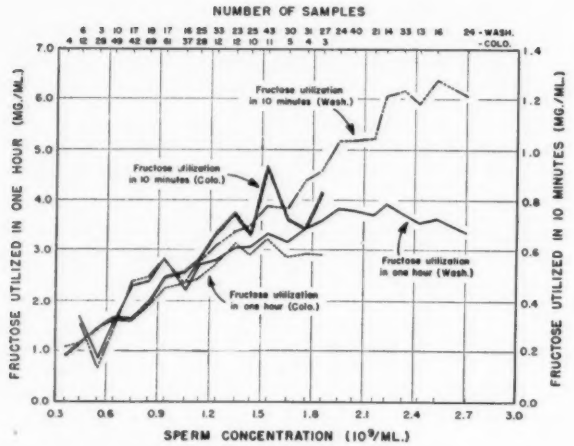
NUMBER OF SAMPLES
24 - WASH.
- COLO.
FRUCTOSE UTILIZED IN ONE HOUR (MG./ML.)
FRUCTOSE UTILIZED IN 10 MINUTES (MG./ML.)
Fructose utilization in 10 minutes (Wash.)
Fructose utilization in 10 minutes (Colo.)
Fructose utilization in one hour (Wash.)
Fructose utilization in one hour (Colo.)
SPERM CONCENTRATION (10⁹/ML.)

FIG. 1. Comparison by states for fructose utilization during 10 minutes (calculated) and 1 hour of incubation at 37° C. (Two samples were above the ranges shown for Colorado.)

The correlation between sperm concentration groups (means) and fructose utilization during 10 minutes was $r = 0.98$ ($P < 0.01$) for Washington and $r = 0.94$ ($P < 0.01$) for Colorado. The regression coefficients of 0.49 and 0.45 mg. of fructose for 10 minutes incubation for each increase in 10^9 sperm per milliliter for Washington and Colorado, respectively, were not significantly different.

Plotting fructose utilization rates against concentration (Figure 1) by states

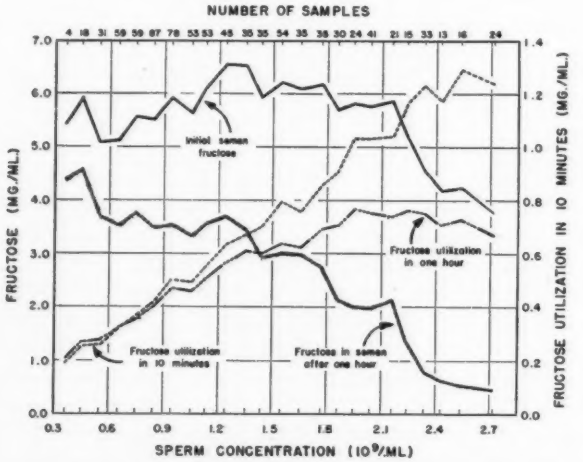
NUMBER OF SAMPLES
FRUCTOSE (MG./ML.)
FRUCTOSE UTILIZATION IN 10 MINUTES (MG./ML.)
Initial semen fructose
Fructose utilization in one hour
Fructose utilization in 10 minutes
Fructose in semen after one hour
SPERM CONCENTRATION (10⁹/ML)

FIG. 2. Relation between sperm concentration and utilization of fructose during 10 minutes (calculated) and 1 hour of incubation at 37° C.

shows the consistency of fructolysis in the two sets of data. The linearity of fructose utilization during 10 minutes incubation is similar for each state and utilization during 1 hour decreases with increasing sperm concentration.

Influence of concentration. Several important factors influencing fructolysis rate are apparent for the combined data (Figure 2), namely: *(a)* Initial semen fructose reached maximum levels in 80 semen samples containing between 1.2 to 1.4 $\times$ 10^9 sperm per milliliter and declined steadily with further increases in concentration; *(b)* the linear relationship between concentration and fructose utilized during 1 hour is lost at concentrations exceeding 10^9 sperm per milliliter; and *(c)* maximum utilization of fructose as estimated for 10 minutes incubation is linear with concentration up to 2.6 $\times$ 10^9 sperm per milliliter. Data in Figure 2 clearly demonstrate that *(a)* lower initial fructose is associated with above average sperm concentration, *(b)* incubation periods of 1 hour are too long for measuring and comparing the fructolytic capacity of semen samples, whereas *(c)* fructolysis rates based on the calculated utilization by 10^9 sperm per milliliter during the first 10 minutes of incubation largely eliminate the discrepancy due to association of inadequate fructose and high sperm concentration.

Level of seminal plasma fructose and utilization rate. Fructose in the whole semen was changed to a value estimated for semen less sperm cell volume *(9)*, which will hereafter be referred to as seminal plasma fructose.

Fructose plotted against initial levels of seminal plasma fructose (Figure 3) shows maximum utilization at 1 hour for 134 semen samples with initial plasma levels between 7.0 and 8.0 mg. per milliliter of semen. However, maximum utilization occurred at 10 minutes for 196 samples with initial levels of 5.0 to 6.0 mg. per milliliter, which is associated with an average substrate concentration of plasma fructose of 2.1 mg. per milliliter at 1 hour; utilization per 10^9 sperm in 10 minutes is also maximal at this level, whereas the hourly rate per 10^9 sperm

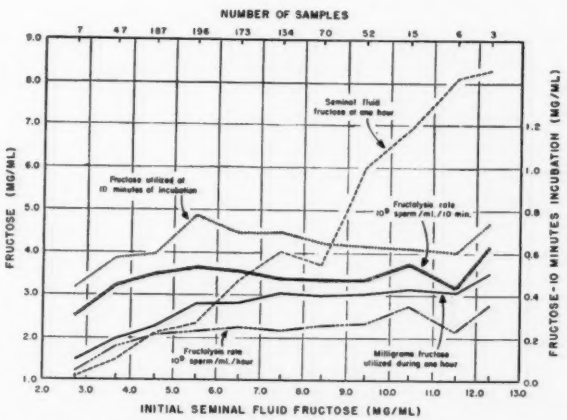

FIG. 3. Relation between seminal plasma fructose before and after incubation and maximum utilization rate by 10^9 sperm/ml. (Eight semen samples were above these ranges.)

TABLE 2

Relation of fructolysis measures with nonreturns using bull means

Source	D.F. (n – 1) Bulls	Av. services per bull	Average[a] 60-90 day NR	Linear correlation (r) – Nonreturn (NR) × Fructose utilization			
				mg/hr	mg/10 min.[e]	mg/10[9]	mg/10[9]
(State)	*(No.)*	*(No.)*	*(%)*	*(r)*	*(r)*	*sperm/hr (r)*	*sperm/10 min.[e] (r)*
Colorado—1st yr.	16	376	63.9	0.64[d]	0.61[d]	0.87[d]	0.85[d]
Colorado—2nd yr.	14	326	60.3	0.87[d]	0.84[d]	0.57[c]	0.75[d]
Colorado—3rd yr.	11	258	58.7	0.84[d]	0.78[d]	0.91[d]	0.84[d]
Colorado—total[b]	43	327	61.2	0.74[d]	0.68[d]	0.69[d]	0.73[d]
Colorado—years[b]	2	—	—	–0.72	–0.83	–0.33	–0.65
Colorado—within years[b]	41	—	—	0.79[d]	0.75[d]	0.76[d]	0.81[d]
Colorado—bull totals	28	496	61.3	0.76[d]	0.71[d]	0.77[d]	0.80[d]
Washington—bulls[b]	35	530	71.1	0.40[c]	0.44[d]	0.06	0.40[c]
Washington—breed	3	—	—	0.93	0.01	0.36	0.91
Washington—bulls within breed	32	—	—	0.38[c]	0.69[d]	0.02	0.37[c]
Washington and Colorado—Bulls (Washington = 36; Colorado = 29)							
Total[b]	64	515	66.7	0.51[d]	0.65[d]	0.17	0.52[d]
State[b]	1	—	—	1.00	1.00	–1.00	1.00
Bulls within state[b]	63	—	—	0.20	0.45[d]	0.47[d]	0.62[d]

[a] Bull means.
[b] Covariance analysis.
[c] Significant at 5% level of probability using $n - 2$ degrees of freedom.
[d] Significant at 1% level of probability using $n - 2$ degrees of freedom.
[e] Estimated from 1-hour utilization based on first order reaction formula.

is highest at initial levels in excess of 10.0 mg. per milliliter. Figure 3 shows that a reasonable level of fructose is required to sustain maximum fructolysis with no evidence that high levels are deleterious. The lower limit of plasma fructose necessary for maximum utilization may be nearer 1.0 mg. per milliliter as observed by sorting the data on the basis of plasma fructose remaining after 1 hour of incubation.

Fructolysis and nonreturns by states. Too few breedings per semen sample prohibited comparison of all 898 samples in the study on a per sample basis *(10, 13, 15, 17)*. In order to directly compare Washington and Colorado fertility data, bull means were used. The Colorado data, analyzed by years (Table 2), showed consistently highly significant correlations between fertility of the bulls and the four measures of fructolysis employed in this study. The estimated 10-minute utilization and utilization rate were superior for 2 of the 3 years. When influence of years was removed by methods of covariance, the utilization by 10^9 sperm per milliliter for 10 minutes was the superior measure.

Some bulls were studied for more than 1 year. The study included 29 bulls with 44 bull-years. However, analyzing on a bull basis changed the correlations for the Colorado data only slightly.

The Washington data (36 bulls) were first analyzed as a unit (Table 2), removing the influence of breeds. The correlations for nonreturn compared with the measures of fructolysis on a bull-within-breed basis were lower than the Colorado comparisons but significant for all but utilization by 10^9 sperm per milliliter per hour. Combining the 65 bulls involved in the two studies showed highly significant relationships for bulls within states for all fructolysis measures except fructose utilized during incubation for 1 hour.

Analyses of variance revealed (Colorado) no significant differences between years for nonreturn or the four measures of fructolysis. The combined data showed Washington significantly higher ($P < 0.01$) for nonreturns, 1-hour utilization of fructose, and estimated 10-minute utilization. Colorado data showed a significantly higher ($P < 0.05$) average utilization by 10^9 sperm per milliliter per hour. There was no difference between states for utilization by 10^9 sperm per milliliter for 10 minutes.

Between-states differences in correlations shown in Table 2 can probably be attributed to differences in the fertility level of the bulls. The ranges in nonreturns for Colorado were 52 to 73, 43 to 69, and 39 to 73%, respectively, for the first, second, and third years. The range in nonreturns for 29 bulls at Colorado observed one or more years during the 3-year period was 50 to 73% compared with 59 to 81% for the 36 Washington bulls.

Sample within-bull relationships. From the Washington data, 400 semen samples were used for 20 or more first and second services. By covariance analysis (Table 3), no critical relationship between nonreturns and fructose or fructolysis existed on a sample within-bull basis.

Initial levels of semen and plasma fructose on a between-sample basis were negatively correlated with nonreturn ($P < 0.01$), as previously observed *(8)*. Ten-minute utilization and rate for 10^9 sperm per milliliter per 10 minutes were

TABLE 3

Comparison of 400 semen samples from the Washington data for relationships between fructolysis measures, concentration, and nonreturns

Linear correlation (r)	Total	Breeds	Bulls	Within bulls
(Comparisons)	*(r)*	*(r)*	*(r)*	*(r)*
Nonreturn × initial fructose	−0.17[a]	−0.73	−0.39[b]	0.02
Nonreturn × initial plasma fructose	−0.17[a]	−0.68	−0.38[b]	−0.01
Nonreturn × 1 hr. level of fructose	−0.21[a]	−0.91	−0.48[a]	−0.00
Nonreturn × 1 hr. level of plasma fructose	0.00	0.01	0.00	0.00
Nonreturn × utilization in one hour	0.14[a]	0.90	0.39[b]	0.02
Nonreturn × utilization in 10 min.	0.19[a]	0.99[a]	0.49[a]	0.02
Nonreturn × utilization 10^9 sperm/hr.	−0.01	−0.20	−0.12	0.05
Nonreturn × utilization 10^9 sperm/10 min.	0.12[b]	0.88	0.26	0.04
Nonreturn × sperm concentration	0.09	0.97[b]	0.23	−0.05
Concentration × initial fructose	−0.52[a]	−0.88	−0.46[a]	−0.43[a]
Concentration × initial plasma fructose	−0.39[a]	−0.86	−0.33	−0.24[a]
Concentration × 1 hr. level of fructose	−0.68[a]	−0.98[b]	−0.61[a]	−0.61[a]
Concentration × 1 hr. level of plasma fructose	−0.64[a]	−0.98[b]	−0.57[a]	−0.57[a]
Concentration × utilization in one hour	0.50[a]	0.72	0.64	0.42[a]
Concentration × utilization in 10 min.	0.67[a]	0.95[b]	0.77[a]	0.53[a]
Concentration × utilization 10^9 sperm/hr.	−0.57[a]	−0.45	−0.72[a]	−0.43[a]
Concentration × utilization 10^9 sperm/10 min.	−0.09	0.70	−0.36[b]	−0.12[b]
Degrees of freedom $(n-1)$	399	3	31	365

[a] Significant at 1% level of probability $(n-2)$.
[b] Significant at 5% level of probability $(n-2)$.

positively correlated ($P < 0.01$) with nonreturns. The slight negative relationship for utilization by 10^9 sperm per milliliter per hour shows again very plainly the influence of inadequate fructose to support fructolysis for an incubation period of 1 hour.

Correlations between sperm concentration and the fructolysis measures (Table 3) verify the results shown in Figure 2; of special interest is the higher negative correlation between initial fructose and concentration as compared with plasma initial fructose. On a sample-within-bull basis the former relationship accounts for 18.8% of the variance due to regression as compared with 5.8% for the latter. This shows that the correlation between sperm concentration and fructose level of semen is due more to varying seminal fluid volume than to differences in seminal fructose concentration between samples from the same bull. This confirms the observation of Mann and Lutwak-Mann *(24)*. The correlation between sperm concentration and 1-hour levels of fructose is considerably higher than initial levels on a sample within-bull basis where the difference between initial and 1-hour levels estimates the influence of three factors within bulls of high fertility, namely, *(a)* low concentration associated with slightly higher plasma fructose and lower utilization during 1 hour of incubation, *(b)* high concentration and inadequate fructose in the incubate to support maximum fructolysis during incubation, and *(c)* samples with below average fructolytic ability from the same bull.

The estimated utilization by 10^9 sperm per milliliter for 10 minutes shows only a slight but significant negative correlation with concentration. This demonstrates that the variation associated with concentration can be removed by short-

ening the incubation period and that utilization rate is directly proportional to sperm present in semen from the same highly fertile bull.

This does not preclude wide differences between bulls ($r = -0.36$) or even between samples from the same bull if that bull is of questionable fertility.

Analysis of variance for breed and bull differences in the data shown in Table 3 showed breeds and bulls within breeds highly significantly different for sperm concentration, levels of initial semen, and plasma fructose and for the four measures of fructolysis. Nonreturns were significantly different for breeds and highly significantly different for bulls within breeds.

GENERAL DISCUSSION

It is unfortunate that an incubation period shorter than 1 hour was not used for the Washington study. However, at the start of the study incubation periods up to 3 hours were being used. Preliminary Washington work failed to reveal that a 1-hour incubation would prove too long for samples with high sperm density. Volume of semen available for experimental use at the breeding association prevented modification of the experiment once it started. The estimated utilization at 10 minutes by use of the first order reaction formula *(17)* was selected for ease of calculation. Though any other time could have been used, the relationships observed would have remained unchanged. Incubation periods involving too little time could induce errors by masking important differences between semen samples due to *(a)* increased error caused by a combination of low utilization and errors in the chemical determination of fructose (assuming error the same for large changes), and *(b)* slightly variable times required to plateau the enzymatic systems involved because of variations (1) in incubation technique; (2) in the enzyme system characteristics of individual semen samples, and (3) time from collection to start of incubation. Further experimentation is needed for clarification.

Sperm concentration shows no consistent correlation with fertility when semen from bulls of acceptable fertility was used. The semen of a bull before being used by artificial breeding associations is judged for motility characteristics and to a lesser extent for sperm density. Therefore, it is not surprising under these conditions that motility and sperm concentration show little or no relationship with fertility.

Fructolysis rates for semen selected for above-average motility from bulls of known acceptable fertility have shown promise in further estimating differences between bulls of high fertility. The relationships between sperm concentration, initial fructose, and fructose utilization are high enough to be major contributing factors in interpretation of metabolic studies, as has been clearly shown in this study. Differences in bulls, procedures, and length of experimental periods add further to discrepant results.

Since fructose is primarily a constituent of accessory gland fluids *(11, 12, 22, 24)*, a true relationship between sperm concentration and fructose in semen cannot be expected. This has been demonstrated through castration and vasectomy *(11, 12, 14, 16, 22, 24)* experiments. Further differences may occur because

of incompleteness of the ejaculatory response, causing variations in the proportion of accessory gland fluids and sperm. Adjusting semen fructose levels to a seminal plasma basis *(9)* reduced threefold the negative relationship between fructose initially present and concentration.

Greater sperm concentration reduces plasma volume and thereby reduces the fructose per milliliter of semen. Other studies *(2, 15, 17-19, 24, 25, 27)* show that utilization during incubation is on the average directly related to sperm concentration. This study shows the relationship to be a linear one. Therefore, semen samples with above average sperm concentration have below average fructose per milliliter of semen and above average fructolysis during incubation; fructose becomes a limiting factor when sperm concentration is high and the incubation period is too long. By-products of fructolysis apparently do not influence utilization rate through the range shown in Figure 2, though a combination of adequate fructose and longer incubation may show otherwise.

Owing to the interrelated nature of fructose and sperm concentration, incubation periods for determining maximum fructolysis rates should be considerably less than 1 hour when using semen of very high quality. Taking into consideration the techniques and biological aspects of the problem, an incubation period of 20 to 30 minutes is suggested, with preference being given to the former. When quantity of semen is not a limiting factor, making fructose determinations at more than one time during incubation is preferable *(17)*.

SUMMARY

Data from Colorado and Washington, involving 898 semen samples, were combined to study the relationship between fructolysis, incubation intervals, sperm concentration, and fertility.

An incubation period of 1 hour at 37° C. was found to be too long for proper estimation of fructolytic capacity on a 10^9 sperm basis. Estimating utilization at 10 minutes by use of the first order reaction formula showed that: *(a)* fructose utilization is directly proportional to the number of sperm in the incubate (1:4 dilution with citrate or 2:1 dilution with phosphate); *(b)* correlation with fertility is improved, particularly for semen with the higher average concentration.

By using bull means (65 bulls from Colorado and Washington) the bull within-state correlations between nonreturns and fructose utilized during 10 minutes (estimated) and 1 hour of incubation and utilization by 10^9 sperm per milliliter for 10 minutes and 1 hour were found to be, respectively, 0.45, 0.20, 0.62, and 0.47 ($P < 0.01$ except for $r = 0.20$).

Four hundred semen samples from the Washington data showed no relationship between nonreturns and any of the fructose measurements for semen samples within bulls. With proper recognition of interfering variables, fructose utilization by 10^9 sperm per unit time is a satisfactory laboratory method for estimating fertility of bulls. An incubation time of 20 minutes at 37° C. is suggested for highly concentrated, highly motile semen. Dilution of the incu-

bates in this study was with 2 parts semen and 1 part phosphate and 1 part semen and 4 parts citrate.

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TECHNICAL NOTES

THE EFFECT OF FAB AND LECITHIN ON THE DIGESTIBILITY OF AN ALL-HAY RATION

The successful use of certain of the common household detergents in the prevention of bloat in cattle has recently been reported *(1, 2)*. Some evidence has also been obtained which indicates that soybean lecithin may be effective in reducing the incidence of bloat *(3)*. The latter product would seem to hold the most promise as a bloat preventative since it is readily eaten by livestock, whereas the detergents are unpalatable.

It is conceivable that these products, although effective in preventing bloat, may adversely affect the microorganisms of the reticulo-rumen and thus lower the digestibility of the feed. The purpose of the work reported here was to determine the effect of orally administered Fab and purified soybean lecithin on the dry matter digestibility of the ration.

Six Holstein heifers weighing from 450 to 660 lb. each were fed an alfalfa–oat hay mixture as the basal ration. The mixture was predominantly oats and was fed twice daily in amounts that would result in a minimum of refused feed. The basal ration was supplemented with either Fab or lecithin, each fed at two levels. The levels of Fab were 10 and 20 g. daily given in capsule form (one-half of total amount given at each feeding). The lecithin was heated and poured over the hay at each feeding in amounts equal to 1 and 2% of the hay intake (air-dried basis).

A 3 × 3 Latin square design was used to test each level of Fab and lecithin feeding. The experimental periods were 18 days in length, consisting of a 12-day preliminary period and a 6-day period during which total fecal collections were made. The amount of hay to be fed during each collection period was determined prior to the start of the fecal collections and core-sampled for moisture analysis.

The daily hay intake averaged 12.1 lb. per animal with a range of 10.4 to 14.0 lb. Thus, the lecithin intake varied from approximately 0.1 to 0.3 lb. per head daily.

The dry matter digestion coefficients are summarized in Table 1. It will be noted that the control rations and the rations containing lecithin were equally digested, whereas the digestibility of the hay was decreased when Fab was fed at either the low or high level. An analysis of variance revealed, however, that this decrease was not statistically significant.

TABLE 1

Average dry matter digestion coefficients

	(%)
Low level	
Hay + Fab	54.9 ± 0.77[a]
Hay + lecithin	56.7 ± 0.59
Hay only	56.9 ± 0.25
High level	
Hay + Fab	54.2 ± 1.00
Hay + lecithin	56.5 ± 0.82
Hay only	56.2 ± 1.04

[a] Standard error of the mean.

It would appear from these data that the feeding of Fab and purified soybean lecithin is without detrimental effect to the reticulo-rumen microorganisms or dry matter digestibility of the ration. However, the detergents may be expected to lower digestibility slightly.

Although these results were obtained under conditions where bloat is not frequently encountered, it seems logical to assume that they would apply to the free grazing animal or to animals hand-fed green herbage.

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OUR INDUSTRY TODAY

Brief Reviews of Current Topics

Recommended Policies for a Sound Dairy Program

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In any discussion of policies for a sound dairy program three important considerations must be kept in mind.

1. The dairy industry is an integral part of American agriculture, and the economic position of dairy farmers cannot be long maintained at a level far different from that of other farmers.

2. The dairy industry, although vitally affected by foreign trade, is primarily a domestic industry.

3. The dairy industry has many unique problems which require specialized consideration of dairy farmers themselves.

A broad look at all agriculture is helpful in any discussion of problems facing dairy farmers. It doesn't take much of a crystal ball to lead one to the conclusion that all is not well in agriculture and that some policy changes are in order—if the family farm is to remain a cornerstone of American democracy.

Net cash farm income in 1955 dropped 38% from 1948, and 32% from 1951. During these periods national income has soared to greater and greater heights. The cost-price squeeze has affected all farmers.

The agricultural price problem grew from an expansion in production beyond demand for peace-time requirements. Agricultural production was built up in response to heavy wartime and postwar needs. Consequently, as wartime outlets vanished, surplus food and fibre sent agricultural prices downward. Agricultural production cannot be turned off like a faucet. Technological progress cannot be turned back. Neither can land be retarded easily from useful production.

There has been an increase in production per cow resulting from artificial insemination programs, better feeding, and better herd management. Large sections of the country have converted from the production of farm separated cream to the marketing of whole milk with the consequent development of a large nonfat dry milk solids industry. We are currently witnessing more changes with bulk tank milk handling on the farm. These trends cannot be reversed, and to attempt to do so would be doubtful public policy.

The level of support prices in agriculture served as the scapegoat for postwar surpluses. They were blamed for inducing unnecessary production and for pricing ourselves out of markets for farm products at home and abroad. The prescribed remedy was lower support prices. The lower prices were to increase demand and shrink agricultural production, or to shift production from products in over-supply to those which offered better income opportunities.

Lower Support Prices Have Not Been the Answer

One cannot dispute the theory that higher prices will encourage production, and that lower prices will discourage production, but the problem is not that sample.

The dairy industry was the first major segment of agriculture to feel the lowering of the boom on price. The support level on manufacturing milk was dropped from $3.74 to $3.15 per hundredweight April 1, 1954. When this adjustment was made, it was predicted that milk production would drop, consumption would increase, and that dairy farmers would benefit from the lower prices. In the adjustment, however, dairy farmers had little opportunity to switch from the production of milk to other commodities. Furthermore, the dairy farmers' creditors often would not permit them to decrease production. Many were thus caught on a treadmill that actually resulted in higher production as a result of the lower prices. Consequently, the production of milk, which in 1953 stood at 121 billion pounds, increased to 124.5 billion pounds in 1955, and the Department of Agriculture now predicts total milk production in excess of 126 billion pounds for 1956. This means a 5 billion pound increase. Lower prices then did not decrease milk production. Nor can it be argued that the reason that milk production did not decrease was because dairy farmers found their position profitable.

The possibilities of expanding outlets for dairy products in foreign markets were not improved when the support prices were reduced. A butter price of 58¼ cents, for instance, is no more competitive than a price of 66½ cents f.o.b. New York when sales are to be made in the world market where butter is sold at prices ranging from 37 to 41 cents per pound, and most of that under bilateral trade agreements.

Nor were per capita sales expanded in the domestic market. In 1952, domestic per capita sales of milk and dairy products amounted to 688 lb. In 1953, per capita sales declined 2% to 674 lb. Since that time we have recouped about half this loss.

The drop in support levels cost dairy farmers $600,000,000 annually. The 1% increase in sales from 1953 to 1955 returned to dairy farmers only $25,000,000 of the $600,000,000. And we cannot be sure that low prices were responsible for the slight regain in consumption. Although per capita sales have changed but little in recent years, our population growth has provided an expanded market for dairy products.

The Soil Bank Plan

At the present time Congress is specifically considering methods of reducing total agricultural production, conserving resources for future generations, and at the same time maintaining income to farmers at least reasonably in balance with the rest of the economy. The most general remedy prescribed is the soil bank program. This program, however, is being discussed in many different forms. The National Milk Producers Federation subscribes to the objectives of the soil bank program but has been quick to point out that unless certain safeguards are written into the plan, it could result in further difficulties for dairy and livestock producers. The recommendations of the Federation regarding the soil bank program are:

1. That the program be voluntary.
2. That payments for soil bank acres be sufficiently high to make them competitive with acres used for agricultural production.
3. That payments for soil bank acres be forfeited if such land is used for grazing or hay.
4. That payments be made in cash and not in surplus grain.
5. That import quotas be used to prevent foreign production from entering U. S. markets to offset any possible increase in prices to U. S. farmers which otherwise might be expected to result from the curtailment of production.

Unless these safeguards are written into the soil bank program, there is little likelihood that farmers will benefit from it, particularly in 1956.

Dairy Farmers Should Aid in Formulating Safeguards for Agriculture

The responsibility for developing safeguards in general agricultural programs and for developing sound legislation, specifically aimed at the dairy problem, rests largely upon the shoulders of dairy farmers themselves. This is because the dairy industry has many unique problems. These involve legislation that can be most effectively presented to Congress by a specialized organization that is intimately acquainted with the peculiarities of the marketing of milk. For dairy farmers the best avenue of approach is through their marketing cooperatives, since the job that farmers are trying to do through their cooperatives involves dairy legislation.

Dairy cooperatives in turn can best make their efforts count through an organization such as the National Milk Producers Federation. As the representative of the dairy farmer and the cooperatives, the Federation concerns itself solely with problems of dairy farmers before the legislative and executive branches of the government. A large measure of its success can be credited to the fact that its members come to grips with the problems of the industry on a day-to-day basis in marketing milk.

It is difficult to visualize, for instance, anyone being more familiar with the effect of legislation, such as the Agricultural Marketing Agreement Act, than the cooperatives operating under the Federal Order program. Nor is anyone more qualified to speak on the level of support prices than the cooperatives whose sole energies are directed toward the problem of obtaining maximum returns to dairy farmers consistent with maximum consumption. Who in the farm group is more familiar with the export problems than the cooperatives in the export business? Who is more qualified to speak on quality regulations than the cooperatives affected by them in their daily operations?

The development of dairy policy involves both short-time and long-time considerations. And in the development of long-time policies, we should be ever mindful that farmers are trying to live today. There are several long-time dairy aims of long standing which are paying off today. These include the development of efficient production and higher production per cow and the development of quality standards and health regulations that have been so important in building public confidence in the industry.

There are at least three other long-term aims in which progress has not been as consistent. These objectives demand attention:

1. The maintenance of prices at maximum levels consistent with the economics of production and consumption.
2. Some system of selling in the world market so that we can compete with other

producers without dragging our domestic price level down to the world price level.

3. The development of the goodwill of the public and good press relations.

Better consumer relations are important to the dairy industry if maximum consumption of milk and dairy products is to be obtained. Perhaps our press and public relations have not been good because when consumers feel impelled to criticize the government price support system, they talk about dairy farmers and dairy products. They don't talk much about a bale of cotton or a bushel of wheat because they have a more intimate relationship with a quart of milk and a pound of butter. As a result, the dairy industry perhaps has received more adverse criticism than any other major segment of American agriculture. Actually, the cost to the government for supporting the farm price of milk and butterfat, relatively speaking, has been a minor item.

The Self-Help Program

As one answer to public criticism, dairy farmers have been pressing for a self-help program for supporting and stabilizing their own industry, without dependence on taxpayers and without the restrictions inherent in a government-operated program. Under self-help, producers could support their own prices at higher levels, manage their own surplus disposal in the domestic and foreign markets, and return more money to U. S. dairy farmers.

Pending the adoption of self-help, the Federation is suggesting that the support level be re-established at $3.74 per hundredweight for the marketing year beginning April 1, 1956, as contrasted to $3.15 as currently in effect.

Other matters before Congress that would implement the policy objectives of dairy farmers include:

1. A revision of the formula for computing the parity equivalent price for manufacturing milk.

2. An extension and expansion of the special school milk program and increased appropriations for the school lunch program.

3. An extension and expansion of the accelerated brucellosis eradication program.

4. An extension and expansion of programs to reduce government holdings of dairy products.

Dairy Industry Goals

In recommending policies for the dairy industry, one must be ever mindful of our goals. The goals in the dairy industry are abundant production of high quality products and low unit costs of production, processing, and distribution. An efficient dairy industry is a well managed industry. This means over-all management of short-time surplus production, as well as good management in the production and in the physical handling of the products. It means management of our over-all agricultural programs so that surpluses are not transferred from other segments of agriculture to dairy farmers.

In a well managed industry, consumers will willingly pay fair prices that will yield a fair living standard to dairy farmers and a fair return on the investment in processing and distribution facilities.

Current Trends in Cheese Manufacture[1]

W. J. Harper and T. Kristoffersen

Department of Dairy Technology, The Ohio State University, Columbus

Since its beginning over 100 years ago in New York, the cheese industry in this country has become increasingly important. With a growing population and a gradual but steady increase in per capita consumption, the future for cheese as a market for milk looks bright. Along with the increase in volume of cheese production has come, also, an increase in the number of varieties. At present most of the major varieties of cheese manufactured in foreign countries are also made in the United States. Although Cheddar is our most important variety, the manufacturing of other types makes up an increasingly important segment of the industry. A comparison of the per capita consumption figures for major varieties since 1948 reveals some interesting trends (Table 1).

TABLE 1

Per capita consumption of cheese in pounds

Year	American	Swiss	Brick	Blue	Italian	Other
1940	4.3	0.4	0.3	0.05	0.3	0.2
1948	5.1	0.5	0.2	0.05	0.4	0.3
1950	5.4	0.7	0.2	0.05	0.5	0.3
1952	5.4	0.7	0.2	0.06	0.6	0.2

Since 1940, the per capita consumption of all varieties of cheese has increased 32%. Between 1948 and 1952, Swiss, Italian, and brick types

[1] The first in a series of articles prepared at the suggestion of the editor on new phases of cheese manufacture, with particular emphasis upon the non-Cheddar varieties. The next article will deal with the technology of cheese ripening.

The purpose of this series of articles is to describe and discuss the manufacture and technology of non-Cheddar varieties of cheese commonly manufactured in this country. Also, some varieties of cheese less known in this country but well accepted in other parts of the world will be included in the hope that this may create an interest in these types. A market for these little known varieties undoubtedly exists. Many of the European segments of the population, both the ones already established and the ones now entering the country, are not able to find the kind of cheese with which they are familiar. Consequently, they may eat less cheese or no cheese at all.

During recent years numerous changes have been taking place that have affected and/or will affect the manufacture and technology of all cheese varieties. Before discussing each cheese variety in detail, it seems essential to obtain a perspective view of the status and future of manufacturing, ripening, and marketing processes. This article is a summation of the current trends in the cheese industry and a discussion of some possible implications of these trends.

of cheese, which comprise 90% of the non-Cheddar varieties, increased in per capita consumption 14.4, 14.1, and 13.4%, respectively.

Most of the non-Cheddar varieties are sold as natural cheese, very little as processed cheese. It has been observed by those in the industry that the per capita consumption of all types of natural cheese has increased about 12%, whereas per capita consumption of processed cheese has dropped about 5%. Much of this increase in natural cheese consumption is due to an increased demand for natural Cheddar cheese. This trend away from processed cheese indicates that the consumers may be changing their eating habits. In the future many more consumers are likely to look for different varieties when selecting cheese for use on the table and for cooking.

Merchandising Methods Have Improved

Merchandising of cheese has undergone tremendous change in the last decade. The supermarkets and special cheese houses have done much to increase the consumer's knowledge of cheese. The recent American Dairy Association survey has shown that consumers do not use certain varieties of cheese chiefly because of a lack of familiarity with these products. Through their attractive displays of consumer packages the supermarkets and special cheese stores have acquainted the consumer with the variety that is available and have greatly increased sales through impulse buying.

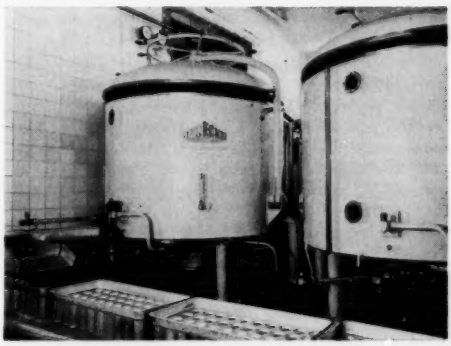
Works Simplification in Cheese Industry

One of the most important trends in the cheese industry is the mechanization of cheese manufacturing practices. Time honored traditional methods are being discarded wherever feasible, and time and labor saving methods are being introduced.

Three major processes stand out today as being of real and/or potential value in shortening the time required for cheese making, the "block" method for making Swiss cheese; the round German cheese making machine, such as Steinecher; and the USDA method for manufacturing Cheddar cheese. Many minor improvements have been or are being introduced in the manufacture of specific types, which will be brought out in the discussions of the individual varieties.

The "block" Swiss cheese process, which will be discussed in a later article, results in a 90-110 lb. block rather than the traditional 180-200 lb. "wheel." This block cheese is an improvement in that it is easier to handle and has less rind. Much of the labor is removed from the process and very little, if any, loss of cheese is encountered in retailing.

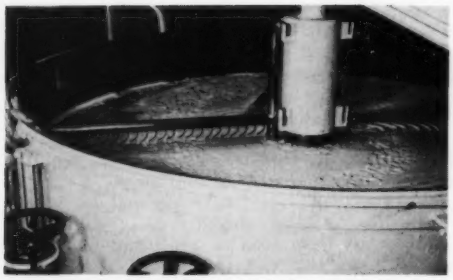
The Steinecher cheesemaker, developed in Germany, was introduced into this country several years ago (Figure 1). Several features



Courtesy Zeiler-Lenz (Freising, Germany)

FIG. 1. Automation in the cheese industry. Camembert forms ready to be filled from the Steinecher cheese making machine. During the operation the drum is under a vacuum and the rate of curd removal is adjusted by controlling the degree of vacuum. By use of such a machine much of the hand labor in filling forms can be eliminated.

make this machine superior to the ordinary cheese vat. The cheese is made in an enclosure, which minimizes possibility of contamination. Special equipment makes the cutting of the curd a mechanical rather than a hand operation (Figure 2). However, the real time and work saver is the drainage device, which makes it possible to empty the curd directly into the hoops without handling. This machine is recommended for certain sweet curd types of cheese and undoubtedly could be adapted for all such types of cheese. For Cheddar cheese a special cheddaring vat would be necessary, though the process might be combined with the USDA



Courtesy Zeiler-Lenz (Freising, Germany)

FIG. 2. A departure from the old style cheese vat. Cutting the curd in a Steinecher vat. The initial curd cutting operation by a curd knife which consists of vertical and horizontal wires in the form of a grid. For soft cheese the frame cuts the curd evenly in one rotation. For hard cheese the frame is moved several times in the same direction, then a curd knife with oblique wires is inserted, and the cutting operation repeated.

cheddaring technique. The use of automatic timer-controls, such as those used for automatic C.I.P. cleaning circuits, could be used to further mechanize the process and to minimize the possibilities of human errors.

The USDA process for Cheddar cheese also illustrates the trend towards mechanization and might be usable for other cheese varieties, such as Romano. The emphasis presently placed on mechanization suggests that further improvements will be made, and a completely continuous cheese making process may be a reality in the future.

Cheese Flavors are Different

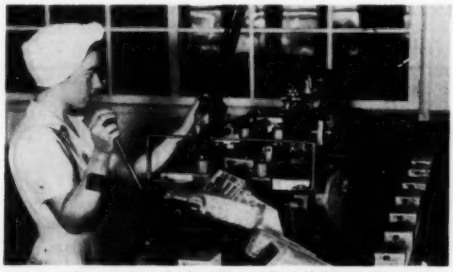
Many of the non-Cheddar cheeses, as well as Cheddar, are losing their original characteristic flavor with the more modern methods of manufacture. The prime purpose of the manufacturing procedure is to bring about a change of the milk into a "medium" suitable for development of the microorganisms necessary to give the cheese its characteristic flavor. Modern mechanized manufacturing techniques in reality come close enough to the techniques of the "old" days, so that they cannot be held responsible for the change that has come in cheese flavor. Pasteurization, modern methods of curing, faster turnover, and modern packaging methods, or a combination of these factors is perhaps the main reason why the flavor of cheese is different from what it was a decade ago.

Pasteurization effect. The change in flavor caused by pasteurization has often been attributed to a change in the bacterial flora of the milk. Perhaps an even greater factor is that pasteurization alters the milk so much that a different "bacterial growth medium" results.

Use of wrappers. Curing the cheese in wrappers of different kinds or in heavy coats of wax to prevent rind formation or to minimize surface growth definitely alters the resulting flavor. The reason has not yet been found but may be related to the diffusion of gases, CO_2 out of the cheese and oxygen into the cheese. Carbon dioxide, being an end-product of certain enzymatic processes, would stop these processes if not removed from the scene of the reaction. The lack of supply of oxygen would stop certain vital oxidative processes in the cheese.

Less aging of cheese today. The marketing of younger or milder flavored cheese perhaps can be related to the general adoption of pasteurization by cheese makers and the making of rindless cheese. The change in flavor brought about by these processes becomes more pronounced as the cheese matures, making early marketing desirable.

The industry has long recognized the problem of obtaining a uniformly aged cheese, and the greater uniformity of the younger mild cheese makes it a more desirable product. A contributing factor is found in automatic packaging, since the body of the cheese is very important to the success of the packaging operation. Generally, aged cheese has less desirable and less uniform body characteristics than young cheese. The cheese industry generally expresses the opinion that the public prefers a mild cheese. Whether the consumer prefers a mild cheese or rather prefers to buy the same flavored cheese each time is a question. The recent USDA survey (USDA 2884-55), which showed a marked preference for sharp cheese in a test survey of two markets, is interesting to note in this regard.



Courtesy of the Olsen Publishing Company

FIG. 3. Automatic packaging machines, such as the one illustrated, permit elimination of tedious hand labor and result in a more sanitary packaged unit.

Together with changes in packaging of cheese and the general procedures of curing and manufacturing has come also increased knowledge of the technology of cheese ripening. Wherever possible in future articles dealing with the specific varieties of cheese, references will be made to recent information on the mechanics and technology involved in the manufacture of these cheeses.

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ABSTRACTS OF LITERATURE

W. O. Nelson, Abstract Editor

ANIMAL DISEASES

179. Oxytetracycline in bovine mastitis. II. Milk levels following local and parenteral administration. L. E. BARNES, Vet Hyg. Dept., Iowa State Coll., Ames. Am. J. Vet. Research, **17**, 62: 18. 1956.

The concentration of oxytetracycline was determined in samples of foremilk collected from treated cows up to 72 hr. after the last treatment. Cows within the milk yield ranges of 10 to 20 lb., 20 to 40 lb., and 40-60 lb. were compared. Treatments were by intramammary infusion of an ointment preparation delivering 400 mg. of oxytetracycline per tube. After single treatments milk antibiotic levels were high at 12 hr., much lower at 24 hr., and almost completely gone at 36 and 48 hr. Three daily treatments with 400 mg. maintained the high levels for the duration of treatment, but the antibiotic disappeared just as rapidly after the last dose as it did after a single dose. 800 mg. treatments established higher levels of the antibiotic in the foremilk, but did not increase persistency. The concentrations of oxytetracycline per ml. of milk were 2 to 10 times as high in the low yielding group as in the higher producers, indicating a need for larger or more frequent doses for high producers in mastitis therapy. Oxytetracycline could not be detected in any of 8 samples of milk taken within 48 hr. after intravenous or intramuscular doses of 1 g. per cow. E. W. Swanson

BUTTER

180. Grade labeling of butter in Wisconsin. H. J. WEAVERS, Dairy and Food Div., Wis. Dept. Agr., Madison. Milk Prod. J., **47**, 1: 20. 1956.

Mandatory grade labeling of butter retailed in Wis. has been in effect since October 1, 1954. This program has stimulated interest in cream and butter quality, butter manufacturing, and packaging procedures.

Butter sold in Wisconsin bears the U.S.D.A. Grade Label, the Minn. Grade Label, and the Wis. Grade Label. The successful operation of such a program requires some provision for reciprocity. J. J. Janzen

181. Butter cutting and dispensing apparatus. G. E. MARKLEY and L. L. GUSTAFSON. U. S. Patent 2,727,306. 11 claims. Dec. 20, 1955. Offic. Gaz. U. S. Pat. Office, **701**, 3: 497. 1955.

A device for slicing blocks of butter into individual-sized portions. R. Whitaker

182. Process for the preparation of butter from high concentrated cream. B. DE GOEDE (assignor to De Nederlandse Centrale Organisatie Voor Toegepast-Natuurwetenschappelijk Onderzoek). U. S. Patent 2,729,563. 6 claims. Jan. 3, 1956. Offic. Gaz. U. S. Pat. Office, **702**, 1: 171. 1956.

A process for making butter without churning, consisting of the steps of mixing butter with cream of high fat content at 17-33° C. until a uniform blend is obtained. Phase reversion occurs and butter is obtained from the cream. R. Whitaker

CHEESE

183. Increase shelf-life of cottage cheese. L. G. HARMON, G. M. TROUT, and M. D. BONNER, Mich. State Univ., East Lansing. Milk Plant Monthly, **45**, 1: 14. 1956.

Storage of cottage cheese at a uniform temperature of 42° F. results in a longer shelf-life. The shelf-life ranged from 5 to 19 days on the samples obtained on the market and from 6 to 23 days on the samples obtained from the plants. The ave. were 11.9 and 15.1 days respectively. The shelf-life of samples stored at 50° F. ranged from 5 to 16 days and ave. 10.9 days and that of duplicate samples stored at 42° F. ranged from 6 to 23 days and ave. 16.5 days.

Samples in which the pH increased possessed an ave. shelf-life of 13.5 days and samples in which the pH decreased had an ave. shelf-life of 16 days. There was a definite relationship between the pH and the type of off-flavors and spoilage. Unclean flavors seldom appeared in low pH samples while musty flavors were more frequent in the low range. Putrid and bitter flavors occurred throughout the pH range but were more common above pH 5.0. Fruity flavors occurred over the entire pH range while an acid or sour flavor was evident in samples having a pH range of 4.52 to 4.92. Surface slime or discolorization occurred through the pH range but was more common above pH 5.0. C. J. Babcock

184. Rindless block Swiss cheese. R. G. TULANE, G. H. THOMAS, and M. RICHARDSON (assignors to the State of Wisconsin). U. S. Patent 2,727,823. 11 claims. Dec. 20, 1955. Offic. Gaz. U. S. Pat. Office, **701**, 3. 626. 1955.

A process is described for making Swiss cheese in block form without a rind. The pressed drained curd is formed in the shape of a block with one side of the six concave with a cavity volume of about 15% of the volume of the block. R. Whitaker

185. Cheese scissors for Italian cheeses. R. T. STAHL (assignor to Swift & Co.). U. S. Patent 2,729,890. 3 claims. Jan. 10, 1956. Offic. Gaz. U. S. Pat. Office, **702**, 2: 266. 1956.

A device for cutting small diameter cylindrical-shaped semi-plastic cheese in smaller sections. It consists of a tubular scissor-like tool with each jaw or cutting portion semicircular in shape. A fluid under pressure, introduced through the handles, escapes through slits in the jaws. R. Whitaker

CONDENSED AND DRIED MILKS; BY-PRODUCTS

186. Process for drying solutions containing crystallizable material, and product produced thereby. P. F. SHARP (assignor to Foremost Dairies, Inc.). U. S. Patent 2,728,678. 8 claims. Dec. 27, 1955. Offic. Gaz. U. S. Pat. Office, **701**, 4: 849. 1955.

A dry milk powder having the following features: particles spherical in shape, protein dispersible in water, and a portion of the lactose in the form of alpha lactose crystals.
R. Whitaker

DAIRY BACTERIOLOGY

187. Observations on the determination of antibiotics in milk. C. K. JOHNS and I. BERZINS, Canada Dept. of Agr., Ottawa. J. Milk and Food Technol., **19**, 1: 14. 1956.

The regular disc assay method for detecting penicillin in milk was comparable to the so-called "2 hour" test. However, the sensitivity of the disc assay was increased by (1) reducing to 4 ml. the volume of seeded agar per plate, (2) refrigerating "planted plates" for 2 to 6 hours, (3) avoiding overnight incubation at 37° C., and (4) using a (0.5 in.) paper disc.

Dornbush's disc assay using *Bacillus cereus* for detecting aureomycin was found to be less sensitive than reported.

Broth cultures of *B. cereus* and *B. mesentericus* were preferable over the spore suspensions. The methylene blue reduction type of test showed excellent sensitivity in detecting aureomycin. H. H. Weiser

188. Antibiotic-resistant organisms in cheese. J. Am. Med. Assoc., **159**, 18: 1788. 1955.

Cheese is suspected as the origin of certain human infections from strains of micrococcus and streptococcus organisms which became resistant to penicillin and dihydrostreptomycin through the use of these antibiotics administered to cattle for the control of mastitis.
F. E. Rice

DAIRY CHEMISTRY

189. Processes for the preparation of inoculating materials for concentrated milk products and for improving the crystallization of lactose in concentrated milk products. T. R. DE VRIES (assignor to De Cooperatieve Fabriek Van Melkproducten Te Bedum). U. S. Patent 2,730,449. 21 claims. Jan. 10, 1956. Offic. Gaz. U. S. Pat. Office, **702**, 2: 407. 1956.

Lactose, in concentrated dairy products, is made to crystallize in small crystals by pulverizing agglomerates of lactose crystals in the product when it is supersaturated to lactose.
R. Whitaker

190. Method of manufacturing a high protein food product and the resulting product. R. A. BOYER (assignor to Swift & Co.). U. S. Patent 2,730,447. 10 claims. Jan. 10, 1956. Offic. Gaz. U. S. Pat. Office, **702**, 2: 406. 1956.

A food product in fibrous form made from protein and a modifying agent and extruded in filaments into a coagulating bath.
R. Whitaker

DAIRY ENGINEERING

191. Collection equipment for spray drying apparatus. F. E. BONNER (assignor to The Commonwealth Engineering Co.). U. S. Patent 2,727,668. 4 claims. Dec. 20, 1955. Offic. Gaz. U. S. Pat. Office, **701**, 3: 587. 1955.

Equipment is described for the collection and packaging of spray-dried products under sterile conditions. R. Whitaker

192. Jacketed food container. J. PIANTANIDA. U. S. Patent 2,727,649. 1 claim. Dec. 20, 1955. Offic. Gaz. U. S. Pat. Office, **701**, 3: 583. 1955.

A device for insulating a milk can against thermal changes. R. Whitaker

193. Bottle capping machine. I. L. WILCOX and N. S. COLE (assignors to Oswego Falls Corp.). U. S. Patent 2,729,379. 6 claims. Jan. 3, 1956. Offic. Gaz. U. S. Pat. Office, **702**, 1: 126. 1956.

Structural details are given for a capping head for applying cover-all or hood caps to milk or other bottles. R. Whitaker

194. Bottle crating and decrating machines. A. G. ENOCK (assignor to Graham-Enock Manufacturing Co., Ltd.). U. S. Patent 2,730,279. 8 claims. Jan. 10, 1956. Offic. Gaz. U. S. Pat. Office, **702**, 2: 365. 1956.

Bottles to be cased are conveyed to a marshalling table where they are arranged in the same pattern as in the case. A group of gripping devices pick up the bottles and lower them into a case which has been conveyed to a designated place beneath the suspended bottles. The apparatus, when operated in reverse, will remove bottles from cases and deliver them to a moving belt. R. Whitaker

DAIRY PLANT MANAGEMENT AND ECONOMICS

195. Increasing price spreads — Who's to blame? H. MOORE, Univ. of Wis., Madison. The Milk Dealer, **45**, 4: 54. 1956.

A Wis. Legislative Joint Committee recently reported that marketing margins, the difference between farm and retail price in Wisconsin's 8 major fluid milk markets rose from 51% to 59% between 1951 and 1954, while the farmer's share of the consumer's dollar fell from 49% to 41%. During this period great differences occurred in marketing margins between fluid milk markets. The report concluded that about 79% of the increase in gross margin for the fluid milk distributors was attributable to increased operating expenses and 21% to increased profits. However, with net margins averaging 2.9¢ per dollar sales before income taxes, monopoly profits cannot be considered an important factor. It concluded also that marketing margins alone give little evidence as to what is happening in monopoly profits.
C. J. Babcock

196. Measure receiving efficiency. W. M. ROBERTS and J. P. NELSON, N. C. State Coll., Raleigh. Milk Plant Monthly, **45**, 1: 37. 1956.

A method is demonstrated whereby a manager can evaluate the effect of equipment and methods on labor requirements in receiving operations. Further projections would enable him to determine the benefits to be derived from using additional personnel for receiving milk.
C. J. Babcock

197. Merchandising dairy products in Alabama retail food stores. E. K. KIRKWOOD and J. H. BLACKSTONE, Ala. Agr. Expt. Sta., Auburn. Bull. 294. 1955.

A detailed study was made of the merchandising methods, costs, and returns of the 1952 operation of 147 retail food stores in Ala. The purpose of this study was (a) to determine methods followed, services performed, volume of dairy products handled per store, and the relationship of dairy product sales to total sales; and (b) to relate these factors to the costs and efficiencies of retailing dairy products under different circumstances.

The 147 stores varied in sales from $2,948 to $1,250,00 annually. The average was $65,-360. The % of total sales volume represented by the different commodities varied considerably. The ave. of all stores showed groceries contributing 37.4%, meats 25.6%, bakery products 10.2%, produce 9.3%, fluid milk 5.8%, other dairy products 6.5%, non-foods 3.9%, and frozen foods 1.3% of total sales. Fluid milk products ranked second only to meats in sales volume per sq. ft. of display space. However, in amount of gross profit per sq. ft., milk ranked fifth. Meats and fluid milk products ranked highest in efficiency of space utilization. Meats contributed 25.6% of total sales and occupied 8% of the display space. Fluid milk constituted 5.8% of sales and occupied 2.6% of total display space.

All dairy products including milk made up 12.3% of total sales. Evaporated milk ranked second to fluid milk in total sales value; cheese was third. Approximately 45% of all whole milk bought in the area was sold by retail stores. More cream line than homogenized milk was sold in each store. Buttermilk was the only fluid milk product handled in all stores. Approximately 90% of all fluid milk was sold in paper containers. Average markup on fluid milk was 2.22¢ per unit. Storekeepers' suggestions as to a fair markup were 2.2¢ to 5.5¢ per quart.
R. Hunt

198. A study of the milk market of Memphis, Tennessee. M. L. DOWNEN, Tenn. Agr. Expt. Sta., Knoxville. Bull. 242. 1955.

This bulletin describes: (1) the economic characteristics of the Memphis market, (2) the functions of the various institutions in the market, and (3) and analyzes the demand for milk and milk products. The population growth was from 356,000 in 1943 up to 488,000 in 1952. Wages increased during the latter part of this survey but sales of dairy products did not increase proportionally. The price of milk changed 18 times while the index of retail food prices changed 118 times. Food prices increased 71% and milk only 63%.

Daily average sales of Class I milk in less than 1 gal. containers ranged from 100,000 to 186,000 qt. equivalent from Jan. 43 to Dec. 52. Homogenized milk, Bulgarian buttermilk, skim milk, and special creams were relatively more important in 1952 than 1943, while the standard milk, extra rich milk, plain buttermilk, chocolate milk, and light cream were less important. Of the Class I sales, 72-87% were in qt. containers. During the last 3 mo. of 1952 sales in ½-gal. jugs jumped from 1% to over 7%.

Seasonal variations of all fluid products was greatest for light and heavy cream. There were two peak sales periods. The spring peak was asociated with Easter and the fresh strawberry season; the Dec. peak with the Christmas–New Year season. This study revealed that changes in price of milk (regular or premium grades) did not effect sales. A 10% change in the price of light and heavy cream resulted in a loss of sales of about 1.4%.
R. Hunt

199. Efficiencies and flexibility in operating butter-and-milk-powder plants. S. A. WALKER, Univ. of Idaho, Moscow. Milk Prod. J., **46**, 12: 18. 1955.

The author discusses the problem of efficient and flexible operation of butter-and-milk-powder plants at length. The following points are considered: (1) building site with respect to the community; (2) planning the building to permit future expansion and versatility; (3) the selection of equipment that can be easily

cleaned, is standard and flexible in use; (4) efficient utilization of labor; (5) maintaining a close check on supply expenses.

The flexibility of butter-powder plants with relation to cheese and/or fluid-milk market also is discussed. The importance of records on the utilization of fluid milk received and the hours of labor required in relation to volume of milk processed is stressed. J. J. Janzen

200. Economic survey of milk marketing in the Purchase Region of Kentucky. C. C. Erwin and J. B. Roberts, Ky. Agr. Expt. Sta., Lexington. Bull. 631. 1955.

The study was made as a means of supplying information which can be used in improving practices involved in mraketing graded milk in the Purchase Region of Ky. The typical farm in Marshall County contains 88 acres while the typical farm in Ballard county has 194 acres. The average size herd of all farms surveyed is about 20 cows. An increase of from 20 to 25 cows per producer is necessary to take full advantage of available farm labor. One of the most important developments affecting production of milk in the Purchase Region has been the extension of the Memphis milkshed into the area. Of all the producers, 28% ship to the Memphis area. However, the Paducah market provides an outlet for well over a half of the graded producers. Probably the greatest problem in the Purchase is low producing cows. With the use of higher producing cows, improved crop and livestock production practices, it seems possible to increase net earnings by three or four times. R. Hunt

201. Milk producers entering and leaving the Detroit market. E. B. Jones and G. G. Quackenbush, Mich. Agr. Expt. Sta., East Lansing. Special Bull. 397. 1955.

Despite the fact that there was an excess of milk in the area during all of 1953, the total number of producers increased 5% (614 producers). The dynamic nature of the market is illustrated by the fact that 1,530 producers entered and 916 left during the year. A sample of producers revealed that 80% previously sold their milk for manufacturing purposes. Of the producers who left the area, ⅔ discontinued production and ⅓ sold their milk elsewhere. The reason for entering the Detroit market was generally for increased income while those who left and discontinued production did so for health reasons. A price gain of about 73¢/cwt is realized between the manufacturing price and Class I price. R. W. Hunt

202. Efficiency factors and changes in milk distribution 1946-1954. S. Johnson and G. K. Brinegar, Storrs Agr. Expt. Sta., Storrs, Conn. Bull. 312. 1954.

Practices and trends on retail and wholesale milk routes of 19 Conn. milk distributors are presented for the years 1946 to 1954. During this period, sales of Class I milk increased 2%. Milk compared to by-products however, increased slightly in importance even though a greater variety of products were offered. Considering the small area surveyed, it was very surprising to note the variations between dealers in the products handled and their relative importance. R. W. Hunt

203. Holder for milk cartons. R. A. Sherlowsky. U. S. Patent 2,730,278. 2 claims. Jan. 10, 1956. Offic. Gaz. U. S. Pat. Office, 702, 2: 365. 1956.

A holder for quart size paper milk cartons made of wire and provided with a handle to facilitate pouring from the carton.

R. Whitaker

FEEDS AND FEEDING

204. The effect of texture on the nutritive value of concentrates for dairy cattle. N. F. Colovos, et al., N. H. Agr. Expt. Sta., Durham. Bull. 419. 1955.

Over a 3-yr. period, 32 digestion and utilization experiments were conducted to determine the effects of texture on the nutritive value of dairy grain mixtures. To determine the effects of the texture of corn and oats, 26 similar experiments were conducted. The results show that the fine mixtures were superior to comparable coarse textured and pelleted feeds in digestible protein and in TDN. Coarse, very coarse, and pelleted feeds were about equal in nutritive value. Ground corn was superior to flaked but crimped oats were superior to ground. There was no difference in pounds of feeds consumed between the fine and coarse concentrate. However, cows fed the fine concentrate gained more in body weight.

R. W. Hunt

205. Effects of preservatives upon red clover and grass forage ensiled without wilting. K. R. Allred, et al., Cornell Agr. Expt. Sta., Ithaca, N. Y. Bull. 912. 1955.

In 1952 and 1953 a red clover and grass mixture was harvested in mid-June and ensiled without wilting in 4 identical 10′ × 10′ tower silos. One silo was filled with untreated forage and the other 3 with forage treated with 70 lb. of molasses, 100 lb. brewers' dried grains, and 9 lb. of sodium meta-bisulfite per ton of silage. The study showed that the feeding value of the 4 silages was similar. Considering the influence of the different preservatives on immature unwilted forage containing more than 50% red clover, it was concluded that none of the preservatives decreased storage losses or improved the feeding value of ensiled material.

R. Hunt

206. A comparison of silage preservatives. O. T. Stallcup, Arkansas Agr. Expt. Sta., Fayetteville. Bull. 557. 1955.

Silages were made from oats, crimson clover, a crimson clover-oat mixture, alfalfa, sweet

clover, and cotton plants. The quality of the forage to be ensiled and the conditions under which it was made into silage had a direct bearing on the nutritive content and palatability of the finished product. Excellent silage was made from oats cut in the late milk stage, crimson clover cut in bloom, a crimson clover-oat mixture, and alfalfa cut in the early bloom stage, when preservatives were used. Cotton plants from which the balls had been picked and sweet clover cut in full bloom stage were high in fiber and low in moisture. As a result, storage losses were high.

Sodium meta-bisulfite, calcium formate, and Kylage were all useful in preserving ensilage made from high-moisture crops such as oats, crimson clover, crimson clover–oat mixture, and alfalfa. These preservatives were most helpful when used on cotton plants and sweet clover harvested in advance stages of maturity. The palatability of silages treated with sodium meta-bisulfite, calcium formate, and Kylage and with such preservatives as whey powder, molatein, blackstrap molasses, and beet pulp varied considerably. The results suggest that the effects of a given preservative may vary, depending on many factors influencing the microbiological fermentation of silage.

R. Hunt

207. High-protein corn as a source of protein for dairy cows. W. B. Nevens, et al., Ill. Agr. Expt. Sta., Urbana. Bull. 586. 1955.

Illinois High Protein and three commercial strains of high-protein corn were compared with U. S. Hybrid 13. When grown on highly productive soil, the percentage of protein in the forage of the high-protein strains was considerably above those of Hybrid 13 forage, but when grown on poor soil, only small differences in protein content were found. R. Hunt

HERD MANAGEMENT

208. Anti-kicking device for cows. O. V. Norenborg (assignor to Aktiebolaget Separator). U. S. Patent 2,727,493. 8 claims. Dec. 20, 1955. Offic. Gaz. U. S. Pat. Office, **701**, 3: 544. 1955.

To prevent a cow from kicking, a harness is described which is strapped over the hind portion of the animal. R. Whitaker

209. Milk transfer unit for automatic milking machine. R. E. Reeve. U. S. Patent 2,727,491. 7 claims. Dec. 20, 1955. Offic. Gaz. U. S. Pat. Office, **701**, 3: 543. 1955.

The milking claw or cluster of teat cups of a pipeline milking machine is suspended beneath the udder of a cow by means of a strap which passes over the cow's back.

R. Whitaker

210. Mechanical operated teat cup. R. Varin. U. S. Patent 2,727,490. 2 claims. Dec. 20, 1955. Offic. Gaz. U. S. Pat. Office, **701**, 3: 543. 1955.

A teat cup is described which is operated by a reciprocating piston. R. Whitaker

211. Teat cup cleaner. M. E. Dauer. U. S. Patent 2,727,269. 5 claims. Dec. 20, 1955. Offic. Gaz. U. S. Pat. Office, **701**, 3: 488. 1955.

Cleaning of milking machine teat cups is facilitated by means of this device, which consists of a set of blades attached to a central shaft. When rotated in the teat cup, the blades scrape the interior walls of the cup.

R. Whitaker

212. Method of cleaning milking machine teat cups. W. M. Harwedel. U. S. Patent 2,730,469. 3 claims. Jan. 10, 1956. Offic. Gaz. U. S. Pat. Office, **702**, 2: 411. 1956.

A device for inserting into milking machine teat cups, which stretches the cup, scrapes the inside surface, and turns the end portion inside out to permit cleaning and inspection.

R. Whitaker

213. Milking apparatus. T. W. Merritt and C. A. Thomas (assignors to Babson Bros. Co.). U. S. Patent 2,730,071. 12 claims. Jan. 10, 1956. Offic. Gaz. U. S. Pat. Office, **702**, 2: 312. 1956.

A reservoir and valve assembly for holding milk delivered by a vacuum-type mechanical milker and discharging same to atmospheric pressure. R. Whitaker

214. Cow washer. R. K. Jansen. U. S. Patent 2,731,300. 4 claims. Jan. 17, 1956. Offic. Gaz. U. S. Pat. Office, **702**, 3: 632. 1956.

A device is described for spray cleaning the udder of cows. R. Whitaker

215. Convertible milking and washing pipeline system. J. S. Kessler (assignor to Ladish Co.). U. S. Patent 2,730,992. 5 claims. Jan. 17, 1956. Offic. Gaz. U. S. Pat. Office, **702**, 3: 552. 1956.

A pump and pipeline system is described which is permanently installed in the barn for moving milk from the individual cow stall to a milk collecting reservoir. The entire system may be cleaned by circulating a cleaning fluid at high velocity, using the same reservoir, pump, and pipeline. R. Whitaker

ICE CREAM

216. Edible container for ice cream and the like. H. A. Toulmin, Jr. (assignor to The Commonwealth Engineering Company of Ohio). U. S. Patent 2,731,349. 10 claims. Jan. 17, 1956. Offic. Gaz. U. S. Pat. Office, **702**, 3: 644. 1956.

Detailed specifications are given for formulating a dextran base material suitable for making cones and other containers for ice cream. R. Whitaker

217. Confection package. B. W. COLMAN. U. S. Patent 2,728,677. 9 claims. Dec. 27, 1955. Offic. Gaz. U. S. Pat. Office, **701**, 4: 849. 1955.

A stick novelty frozen in a heat-sealed polyethylene film bag. R. Whitaker

218. Method for dispensing frozen confections and apparatus for use in same. R. G. TARR. U. S. Patent 2,728,306. 6 claims. Dec. 27, 1955. Offic. Gaz. U. S. Pat. Office, **701**, 4: 758. 1955.

Ice cream, at dipping temperature, in bulk containers is extruded into a hemispherical-shaped mold and then discharged from the mold for direct serving of individual portions. R. Whitaker

MILK AND CREAM

219. Role of ascorbic acid in development of oxidized flavor in dairy products. E. S. GUTHRIE, Cornell Agr. Expt. Sta., Ithaca, N. Y. Memoir 340. 1955.

The author names as possible sources of oxidized flavors phospholipids, proteins, ascorbic acid, and fats. The factors affecting the development of oxidized flavors include ascorbic acid, concentration, daylight, homogenization, metals, oxygen, and temperature. These studies confirmed the theory that ascorbic acid is the most important factor in the development of oxidized flavor. An extensive bibliography is included. R. W. Hunt

220. Container top label. D. G. MAGILL (assignor to American Can Co.). U. S. Patent 2,729,907. 5 claims. Jan. 10, 1956. Offic. Gaz. U. S. Pat. Office, **702**, 2: 270. 1956.

A cover for the top of a Canco paper milk carton, providing protection for the dispensing outlet and plug and space for labeling or advertising. R. Whitaker

221. Dispensing container and blank with improved pouring mechanism. C. R. ALDEN (assignor to Ex-Cell-O Corp.). U. S. Patent 2,731,188. 17 claims. Jan. 17, 1956. Offic. Gaz. U. S. Pat. Office, **702**, 3: 603. 1956.

A description is given for a protected lip-type pour spout for the Pure-pak type of paper milk carton. R. Whitaker

NUTRITIVE VALUE OF DAIRY PRODUCTS

222. Symposium on nutrition in surgery. I. S. RAVDIN, et al., Univ. of Penn. Am. J. of Clin. Nutrition, **3**, 6: 447. 1955.

In the various papers published in connection with this symposium, milk frequently is mentioned as a dietary essential to hasten repair of tissue, replace fluid and electrolyte loss, and to provide the additional protein and mineral needs for the healing of wounds. Homogenized milk is especially recommended for easier fat tolerance. The high milk solids frequently desired can be provided through the addition of dry milk solids to milk or the use of a concentrated milk. In many cases a sterile milk is necessary. The importance of good nutrition in preparing the patient for surgery and for recovery is emphasized. F. E. Rice

223. Infant feeding with homogenized milk. W. M. BLOCK, Cedar Rapids, Iowa. Quart. Rev. Pediat., **10**, 4: 224. 1955.

On the theory that mothers are unhappy with commonly used methods of formula preparation, observations were made on 328 infants with a feeding routine that the author believes to be simpler. Pasteurized, homogenized milk is used from birth. At first it is diluted and sugar added. Boiling the formula is advocated only until the baby is 6 months old. Solid foods are begun when a quart of milk seems not to satisfy, generally 2-3 months. Infant growth in length and weight was average. The feedings were tolerated well, except that the percentage of diarrhea cases was above the national average, but not above the average observed in the author's community. F. E. Rice

224. Vitamin D whole milk. E. H. STEVENSON, Am. Med. Assoc. Council on Foods and Nutrition. J. Am. Med. Assoc., **159**, 10: 1019. 1955.

The council details the "nutritional standards" for "vitamin D whole milk" and "Evaporated Milk with added Vitamin D." These specifications are preceded by a statement on the "Importance of vitamin D milk," which gives credit to vitamin D milk fortification for the fact that "rickets in infants remains rare in the United States today." F. E. Rice

225. Undernutrition in East Africa. R. F. A. DEAN. Pediatries, **17**, 1: 121. 1956.

Protein deficiency is the main causative factor of malnutrition in children which usually appears in the period following breast feeding and before the child is able to digest adult foods. Dry skimmilk or calcium caseinate added to the diet relieves the condition. Dietary soy bean mash cooked 8 to 10 hours proved fairly satisfactory, but the clinical improvement of the children was slower than when they were fed on the milk products, and the regeneration of albumin was slower. F. E. Rice

226. Retrolental fibroplasia. Influence of reduced electrolyte intake on the incidence and severity of the disease. R. HEPNER, et al., Univ. of Texas, Med. Branch, Galveston; and Baylor Univ. Coll. of Med., Houston, Texas. J. Pediat., **48**, 1: 34. 1956.

Premature infants usually are fed cow's milk formulas in preference to human milk. Premature infants also show a high incidence of retrolental fibroplasia (RLF). A cow's milk

formula has higher mineral content than human milk. A study was made of 142 prematures fed the usual cow's milk formulas, and either human milk or cow's milk formula with reduced electrolytes. No apparent difference in RLF incidence or "clinical well-being" was observed in any of the groups. F. E. Rice

227. Fat retention in infants fed breast milk and humanized cow's milk. P. GUILBERT, D. BAKER, and L. A. BARNESS, Univ. of Penn. Hospital, Philadelphia. J. Pediat., **47**, 6: 683. 1955.

Five healthy infants were fed alternately on pooled human milk and cow's milk modified by addition of vegetable fat and lactose to equicalorie and equiprotein content. Daily records were kept of food intake, stools, urine, etc. The fat content of stools was measured and compared with intake. Fat retentions were approximately equal in the two types of feedings as also was the fecal pH. *L. bifidus* generally was present in the stools during periods when human milk was fed. F. E. Rice

228. Vitamin A deficiency in infancy. M. H. BASS and J. CAPLAN, St. Joseph Hospital, New York. J. Pediat., **47**, 6: 690. 1955.

Cases are reported, and symptoms described, of vitamin A deficiency in babies changed from cow's milk formulas to soy bean products. F. E. Rice

229. Serum calcium and phosphorus in premature and full-term infants. A longitudinal study in the first three weeks of life. E. BRUCK and D. H. WEINTRAUB, Univ. of Buffalo School of Med. Am. J. Diseases Children, **90**, 6: 653. 1955.

While a number of factors influencing serum Ca and P were observed, one phase of this study compared the feeding of cow's milk formulas vs. human milk. Blood chemistry changes were followed on 51 premature and 21 full term babies from birth to about 3 weeks. After the fasting period, serum Ca levels rose when human and cow's milk were fed. Calcium levels appeared to fall during the first few days when cow's milk formulas were fed; however, by the end of the first of second week of life Ca levels were normal in almost all cases. The serum P level of infants fed cow's milk formulas rose more consistently and promptly than it did in the human milk fed group. There was no consistent correlation between rise and fall of Ca and P. F. E. Rice

230. The gastrointestinal absorption of unaltered protein in normal infants and in infants recovering from diarrhea. F. L. GRUSKAY and R. E. COOKE, Yale School of Med. Pediatrics, **16**, 763. 1955.

Quantitation of egg albumin passing unaltered from the gastrointestinal tracts into the blood was accomplished by using rabbit antiserum immunological methods. Unaltered protein was found in the blood after the feeding of 1 g. of crystalline egg albumin per kg. body weight. The level was higher than that in the blood of infants convalescent from diarrhea. While cow's milk proteins were not tested, it is concluded that a similar passage of such proteins would take place, so that in the treatment of diarrheas cow's milk should be withdrawn from the diet. F. E. Rice

231. Metabolic studies with pre-adolescent girls. IV. Utilization of calcium. M. A. GLIDDEN, et al., La. State Univ., Baton Rouge. J. Am. Dietet. Assoc., **32**, 1: 29. 1956.

Calcium intake, excretion, and retention were determined for six subjects. Better retentions were observed during periods when nonfat dry milk solids were fed than during calcium lactate feeding periods. The difference was attributed mainly to the additional phosphorus intake when the milk was being fed, which assisted in the absorption and retention of calcium. F. E. Rice

232. Nitrogen, calcium, and phosphorus utilization by 12- to 14-year-old boys. K. DAUM, W. W. TUTTLE, A. GUGEDAHL, H. ROBERTS, and J. SALZANO, State Univ. of Iowa, Iowa City. J. Am. Dietet. Assoc., **32**, 1: 36. 1956.

Retentions were determined with 6 boys on 3 breakfast regimens: (a) no breakfast, (b) cereal and milk, (c) bacon-egg-milk breakfast. Calcium and phosphorus utilizations averaged about the same in all 3 cases. Nitrogen retentions were better in regimens (b) and (c) than (a), with (b) and (c) being about equal. Negative balances were observed in only 3 individual cases: two negative nitrogen balances in regimen (a); one negative phosphorus balance on regimen (b). F. E. Rice

233. Ulcer diets and coronary arteriosclerosis. J. Am. Med. Assoc., **160**, 5: 424. 1956.

The question of whether ulcer patients on high milk diets are more likely to develop arteriosclerosis is answered in the negative. Dietary intake of cholesterol has a less important relationship to its deposition in the blood vessels than the ability of the individual to utilize it. F. E. Rice

234. Comparison of nutritional results with evaporated milk and pooled mother's milk in young infants. A. SINIOS. Ztschr. Kinderh., **75**: 634. 1955. Abstract in Am. J. Diseases Children, **90**, 6: 730. 1955.

Fifty babies fed evaporated milk formulas and 49 fed pooled human milk did not differ statistically in weight gains and incidence of gastrointestinal disturbances. F. E. Rice

235. The treatment of eczema in infants and children. Part IV. L. W. HILL, Boston. J. Pediat., **47**, 6: 648. 1955.

Milk second to egg white gave positive tests in 100 eczematous babies. Lactalbumin is a

much more common cause of allergy than is casein. Lactoglobulin and opalisin are of least importance. Feeding of too much milk and too concentrated mixtures is partly responsible for the syndrome. Soybean milk substitutes will nourish a baby as well as milk over long periods of time. However, these products are likely to cause diarrhea. The author believes that the frequency of milk sensitivity is exaggerated, and that the change to milk-free foods is a trend that has gone too far.

Occasionally a change from cow's to goat's milk is of benefit to a baby sensitive to the former. The reason is not clear since the caseins of the two milks are identical immunologically and chemically, although the two lactalbumins differ somewhat immunologically.

F. E. Rice

236. Allergy in infants and children. N. W. Clein, Univ. of Wash. Modern Med., **24**, 3: 69. 1956.

One baby in 15 is allergic to cow's milk. Symptoms appear between 2 weeks and 2 months. Eighty % of these can tolerate milk after 4 months and about 15% will be allergic after 1 year. Observations on 206 infants appearing to be allergic to cow's milk showed 12 clinical syndromes. Listed in decreasing numbers of cases these are: (1) eczema, (2) persistent pylorospasm, (3) severe colic, (4) diarrhea with mucus and blood, (5) very unhappy, (6) cough, croup, choking, gagging, mucus in throat, (7) constant nose cold, (8) asthma, (9) obstinate constipation, (10) refusal of milk, (11) toxemia with apathy, cyanosis and collapse, (12) urticaria, angioedema. Soybean mixtures, nutramigen, goat's milk, and meat milk are replacements for cow's milk. Orange juice also is mentioned as a "most common allergen in infants and should be avoided the first year." F. E. Rice

237. Galactosemia. Anon. J. Am. Med. Assoc., **160**, 4: 341. 1956.

In some normal infants the failure of the liver to metabolize galactose may be temporary. In chronic cases the defect seems to be permanent; and a milk-free diet is necessary for the remainder of life. F. E. Rice

238. The incidence of milk allergy in pediatric practice. C. Collins-Williams, Allergy Clinic, Univ. of Toronto. J. Pediat., **48**, 1: 39. 1956.

Out of 3,000 cases in private practice only nine cases (0.3%) of definite milk allergy were discovered. The low incidence observed is attributed to the routine use of evaporated milk in prescribing formulas, and the probability that many symptoms attributed to milk allergy reported in the literature are improperly diagnosed. In the few proven cases of milk allergy either a soy product or meat-base formula was substituted successfully for milk. Detailed reports of the nine cases are given.

F. E. Rice

PHYSIOLOGY AND ENDOCRINOLOGY

239. Tocopherol levels of the early milks of semi-range cattle. K. F. Swingle, J. W. Safford, and D. E. McRoberts, Mont. Agr. Expt. Sta., Bozeman. Am. J. Vet. Research, **17**, 62: 28. 1956.

Samples of milk from range cattle from 3 herds which had previously had white muscle disease losses, and from 2 herds which had not had the disease were tested for fat and tocopherols. No cases of the disease occurred during the 2 years of the study, but the herd which had been most seriously affected had the lowest milk concentration of tocopherol. In this herd colostrum and milk tocopherol levels were only 10 to 20% as high as in the other 4 herds. The tocopherol concentrations varied widely due to poor sampling from the wild cows. Colostrum values were generally in the range of 200 to 600 meg. per 100 ml. Milk values from 8 to 32 days were relatively constant at 100 to 150 meg. per 100 ml. The average tocopherol content from 5 dairy cows was 283 meg. per 100 ml. in colostrum and 79 meg. per 100 ml. in 8 to 32 day milk samples. E. W. Swanson

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